DOCTOR OF HEALTH (DHEALTH)

The NutCracker Study

a study of incidental sensitisation to peanut in egg allergic children, and the utility of component-resolved diagnostic testing to Ara h 2 in predicting clinical outcome

Marriage, Deb

Award date:
2017

Awarding institution:
University of Bath

The NutCracker Study:

A study of incidental sensitisation to peanut in egg allergic children, and the utility of component-resolved diagnostic testing to Ara h 2 in predicting clinical outcome

Volume 1

Deborah Elisabeth Marriage

A thesis submitted for the degree of Professional Doctorate in Health

University of Bath
Department for Health

October 2016

Table of Contents

List of Tables

List of Figures

Acknowledgments

First and foremost I would like to thank the study children and families for being willing to participate in this project. I am also indebted to the nurses based on the Clinical Investigation Unit at Bristol Royal Hospital for Children for their help with running oral food provocation challenges.

I would also like to thank The Florence Nightingale Foundation for their financial support towards the completion of this study and, in particular, the Burdett Trust who have kindly contributed funding to help me complete both this thesis and my previous MSc study. I am also grateful to the Royal College of Nursing for their Foundation Bursary.

Huge thanks go to my peers in my PD Health cohort, both near and far, who have all helped to keep me sane through mutual commiseration over hours spent over a keyboard. My family have also helped me keep my feet firmly on the ground, mostly by ignoring the fact that I have been trying to study.

I would also like to thank my supervisors; James Turner for his guidance and attention to detail, and the eminent Professor John Henderson, who has taught me much about the art of research over the last decade. There are many others who have encouraged my journey through education, but in particular I would like to thank Dr Simon Bignall who first enticed me away from ward-based nursing towards academic study, and Professor Gideon Lack who introduced me to the world of allergy.

Abstract

Peanut allergy affects 1-2% of UK schoolchildren. Children with egg allergy are at increased risk. The diagnosis of peanut allergy in this group of children is challenging, with current diagnostic techniques being inadequate. Clarification of peanut allergy status in egg-allergic, peanut-sensitised children is complicated and frequently includes the need for an oral provocation challenge. This places considerable pressure on day-case services, poses a potential risk to the child and carries health economic implications. Recent research has proposed the measurement of specific IgE concentrations to the peanut component Ara h 2 to be a better test for the differentiation of allergy and tolerance than existing methods.

The present study attempts to improve the diagnostic process for this group of children. The primary aim was to investigate the diagnostic value of measuring Ara h 2-specific IgE concentrations in predicting a clinical reaction to peanut. 105 eligible children were prospectively recruited via the tertiary allergy clinic at Bristol Royal Hospital for Children. Children were subjected to a peanut skin prick test and specific IgE testing to whole peanut and Ara h 2 followed by an oral provocation challenge. Children were allocated to either the peanut allergic or tolerant group. Outcomes were related to all three tests.

The peanut skin prick test and whole-peanut specific IgE were poor discriminators between allergy and tolerance. Ara h 2 was the best predictor of peanut allergy, but had greater clinical utility as part of a two-step approach. Receiver-operator curve construction identified optimal cut-off values of 6mm for peanut skin prick testing, 0.39kUA/L for Ara h 2-specific IgE concentrations and 1.08kUA/L for whole peanut specific IgE. These were included in a diagnostic two-step model. When used in isolation, specific IgE concentrations to Ara h 2 were unable to replace the need for an oral provocation challenge for the majority of egg-allergic, peanut-sensitised children.

List of Abbreviations

AUC	Area under the curve
CRD	Component-resolved diagnostics
DBPCFC	Double-blind, placebo-controlled food challenge
IgE	Immunoglobulin E
kUA/L	kiloUnits of specific IgE antibodies per litre
LEAP	Learning early about peanut allergy study
MHC	Major histocompatibility complex
NPV	Negative predictive value
OPC	Oral provocation challenge
PPV	Positive predictive value
PR-10	Pathogenesis-related protein-10
ROC	Receiver-operating characteristic curve
SPT	Skin prick test
SpIgE	Specific IgE

Chapter 1

Introduction

A group of workers take a break during peanut threshing. Durong, 1920-1930.
Item is held by John Oxley Library, State of Queensland.

CHAPTER 1

INTRODUCTION

1.1 The history of human peanut consumption

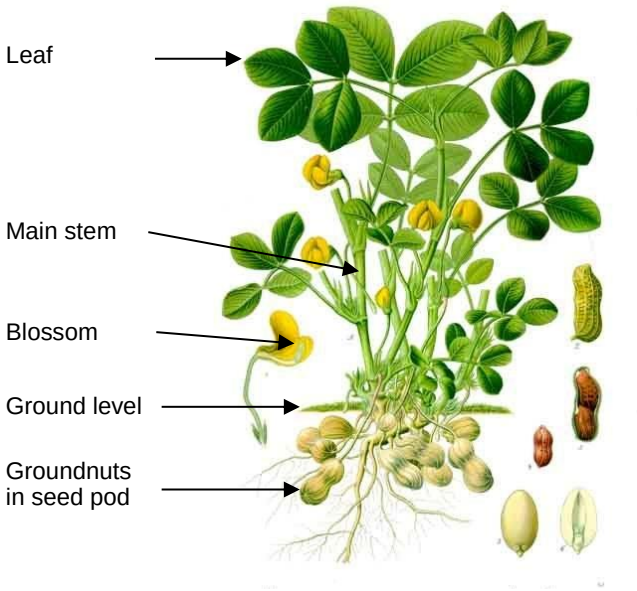
The peanut plant, *Arachis hypogaea*, is commonly known as the groundnut: *hypogaea* literally means 'under the earth'. It is a member of the Fabaceae family (also known as *Leguminosae*) and is native to South America. In the 1500s Spanish explorers carried the plant to Spain, from where it was subsequently traded with Asia and Africa by slaving ships. In the eighteenth century peanuts were transported from Africa back across the Atlantic to North America where they were first grown primarily for livestock (Sauer, 1993). Through the late 19th and early 20th centuries peanut was primarily used to provide animal feed, although after the American Civil War peanuts became a popular high protein food for the army. Popularity further increased in the late nineteenth century when travelling circuses and street vendors began to sell peanuts to the general public. During this period, peanuts were still being harvested by hand. More sophisticated mechanical farm equipment was not developed until the 1900s when an epidemic boll weevil infestation devastating the cotton crop led to the establishment of peanuts as a commercial crop. To a large extent this was due to numerous recommendations by the botanist Dr George Washington Carver for the use of the peanut crop as an alternative to cotton for the benefit of poor farmers. Uses for peanut grew rapidly and included food products, cosmetics, coffee, glue and plastics (McMurry, 1982).

Peanut is an annual herbaceous plant (that is, it has no persistent woody stem above the ground). Botanically, the peanut is not a nut, but a close relative of the legume family, *Leguminosae*, with the mature fruit developing underground in a pod containing up to three seeds as shown in Figure 1. The protein content of the peanut is between 24 and 29%, comprising primarily of seed storage proteins (Koppelman et al., 2001). Legume seed storage proteins comprise the third largest source of dietary protein on Earth (Singh B, 1991). There are four main botanical varieties of peanut plant; Valencia, Virginia, Spanish and Peruvian Runner, the last of which is the dominant peanut variety.

Peanut remains an important global crop with an annual yield of 29 million metric tonnes per year: China, India and the US are the world's three largest producers (APC, 2014). Peanuts are now the twelfth most valuable cash group in the US with a value of over one billion dollars and are an important food crop, with 42 million acres being allocated to peanut cultivation worldwide. Peanut ingestion can cause severe allergic reactions in some individuals, with 1-2%

of UK children having peanut allergy (Sicherer et al., 2010, Venter et al., 2010, Nwaru et al., 2014). Peanut allergy is an adverse immune response that occurs in susceptible individuals.

Figure 1: The peanut plant *Arachis hypogaea*



Legend. Adapted from Kohler, 1887. The mature fruit develops in pods underground.

1.2 Introduction to the human immune system

The human immune system exists to defend the individual and to eliminate foreign substances. It can be considered to have two major branches: innate and acquired immunity, which differ in terms of the specificity and speed of the response, and a memory property. The innate response provides immediate host defence by responding in the same way to all foreign substances, either new, or previously encountered, and is present in all animals. In comparison, the acquired response is highly specific and has a memory property that allows a faster and more robust response if the invading pathogen is encountered a second time (Moser and Leo, 2010).

1.2.1 Innate immunity

The innate immune system comprises a number of processes including phagocytic cells, neutrophils, eosinophils, interferons, natural killer cells and the complement system. *Phagocytic cells*, which include neutrophils and macrophages, engulf and digest foreign organisms at the site of infection; *interferons* are chemicals released to prevent intra-cellular viral replication and are produced by host cells during an acute viral infection; *natural killer*

cells are able to spontaneously kill target cells without prior sensitisation and finally, *the complement system* comprises at least 20 serum proteins which function to activate a cascade pathway to control inflammation. The innate response is able to discriminate foreign cells from self but is otherwise a non-specific system, the activation of which can sometimes lead to tissue damage (Parkin and Cohen, 2001).

1.2.2 Acquired Immunity

The acquired immune response is more sophisticated than the innate response, due to its ability to recognise and remember minor structural components on the surface of foreign organisms. These are known as 'epitopes'. The highly-specific acquired immune response primarily utilises primed *T and B lymphocytes* to recognise and attack antigenic epitopes. B cells can be categorised as *antigen presenting cells*, a term that also applies to dendritic cells and macrophages. All cells of the immune system originate from haematopoietic precursor cells in bone marrow; however B cells develop in bone marrow, whereas T cells migrate from the bone marrow for development in the thymus. Both types of cell have antigenic-binding receptors crucial for successful host defence. Early in cell development, a process of *gene rearrangement* occurs which codes the antigen-binding areas of receptors on the cell surface. B cell receptors comprise four gene segments; the variable (V), diversity (D), joining (J) and constant (C) regions. There are up to 100 V genes, approximately 25 D genes and approximately 50 J genes which assemble at random to form the final VDJ region of the cell receptor. This ensures the production of an almost infinite number of variable receptors necessary for the individual to survive infection by numerous pathogens throughout life (Moser and Leo, 2010). After B cell activation, specific antibody (also known as immunoglobulin) is secreted and produced by plasma cells.

T cell receptors (TCRs) are slightly less complex and exist in two forms with both a constant and variable domain. T cell receptors bind to linear proteins of up to nine amino acids once the antigen has been ingested by antigen presenting cells (usually dendritic cells), processed, and then presented to T cells in the lymph nodes (Parkin and Cohen, 2001).

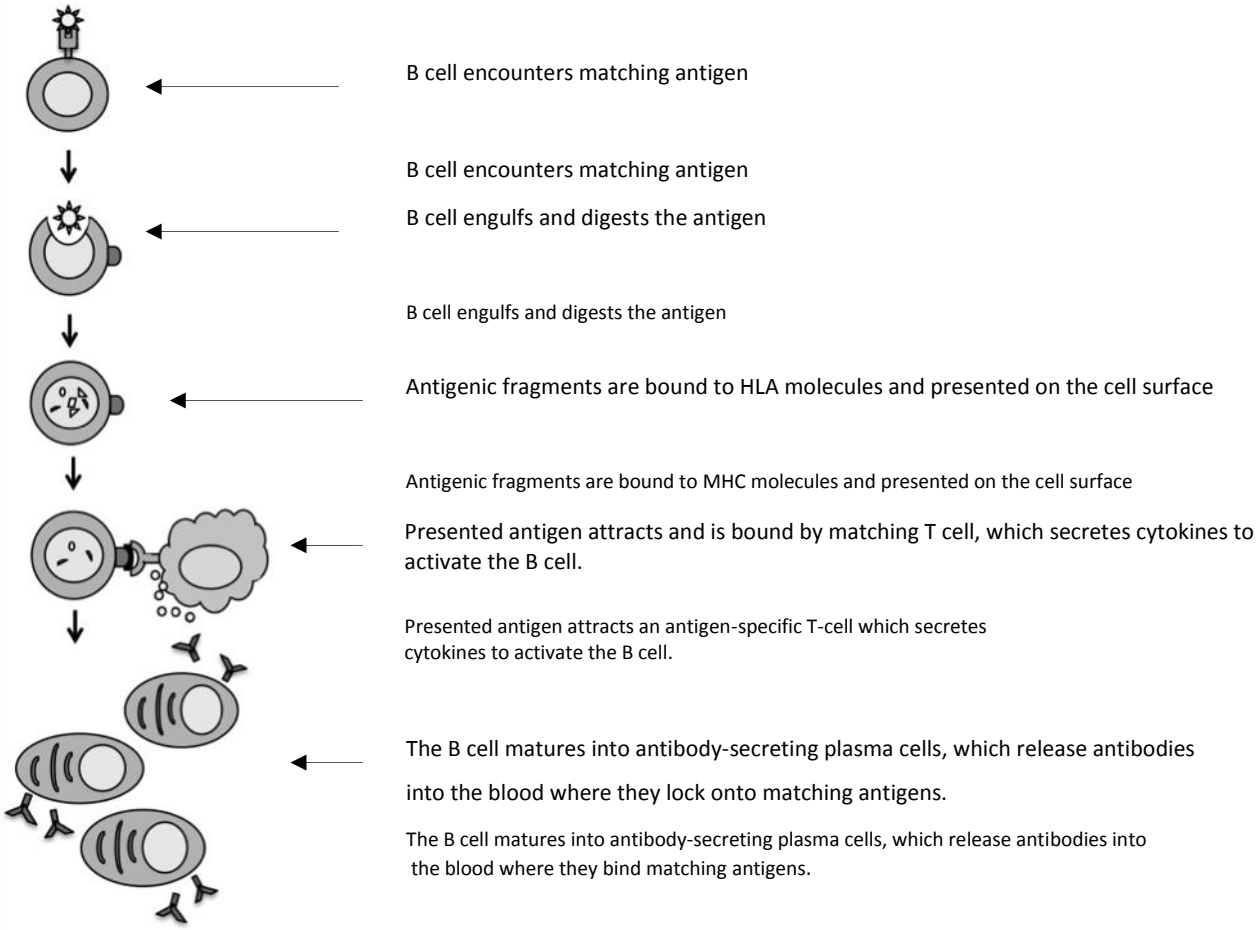
Antigen presenting cells internalise antigens and combine them with major histocompatibility molecules (MHC) which are also referred to as Human Leukocyte Antigen (HLA) molecules. These molecules are moved to the cell surface ready for presentation to T and B cells. T cells are very functionally diverse. There are two subtypes of T cells, T-helper cells which express CD4+ surface molecules and T-cytotoxic (killer) cells that express CD8+ surface molecules. CD4+ T helper cells typically orchestrate the immune response, by production of cytokines that

can assist with antibody production by B cells, whereas CD8+ cells are typically associated with direct killing of infected body cells.

CD4+ T cells are divided into two further functional subsets according to their cytokine production profile (Swain et al., 1991). T helper 1 (Th1) cells produce interleukin-2 (IL-2) and interferon-gamma (IFN-γ) which favours a cell-mediated inflammatory response, whilst T helper 2 (Th2) cells promote a humoral response (that is, soluble immunity via antibody production; *vide infra*) by the production of IL-4, IL-5, IL-6, IL-10 and IL-13. The Th2 response is associated with allergy as these cytokines favour antibody production. IL-4 induces class-switching in B cells which promotes IgE production. Stimulation of IgE production is also brought about by the local environment in which antigen is encountered (for example, gut-associated-lymphoid-tissue that produces high levels of transforming growth factor-beta [TGF-B]) (Nagler-Anderson, 2001). IL-4 also induces further Th2 responses and suppresses Th1 activity (Parkin and Cohen, 2001). T-regulatory cells also exist to modulate the immune response.

The effector functions of T and B cells are brought about by interaction with MHC molecules, which allow the immune system to distinguish between self and non-self. These molecules are subdivided into two major classes, MHC class I and class II. T-cytotoxic cells only recognise antigens which are bound to the MHC class I molecules expressed by all body cells, and which present fragments of foreign proteins, such as those produced by the cell if it is infected with a virus. T-helper and regulatory cells only recognise antigens bound to MHC class II molecules which are only expressed by professional antigen presenting cells (dendritic cells, macrophages, B cells). Antigen recognised by B cells binds to its B cell receptor (a membrane bound antibody molecule; BCR) and is engulfed, processed and presented on MHC class II surface molecules. Antigen-specific CD4+ T-helper cells that recognise the same antigen and have already been activated by dendritic cells, may then recognise antigen presented by B cells in the context of MHC class II. The attracted CD4+ T helper cells up-regulation surface molecules, providing co-stimulation to the B cell and cytokines, which helps the B cell mature and produce antibodies.

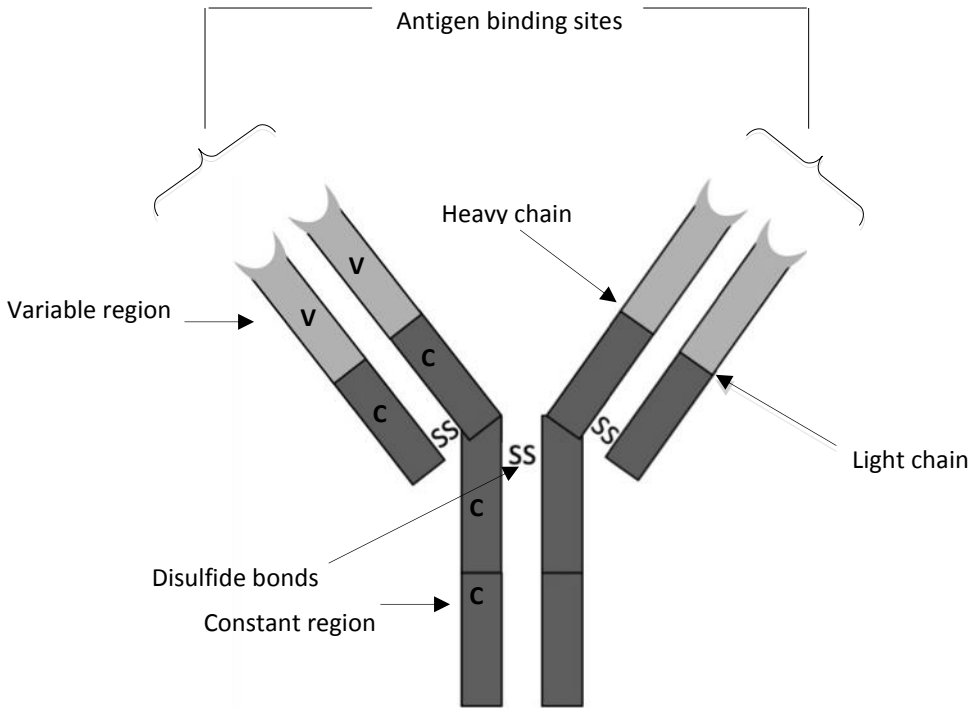
Figure 2: The interaction between T and B cells which leads to B cell activation



Legend. This figure demonstrates the interaction following B cell encounter with a matching antigen until the B cell matures and becomes capable of releasing antibodies into the blood.

Antibodies are Y-shaped molecules which are composed of two identical light chains and two identical heavy chains linked by disulfide bonds. Both chains comprise constant (C) and variable (V) regions (see Figure 3). It is the type of heavy chain which determines the final antibody class or isotype; there are two different light chains (kappa and lambda) and five varying heavy chains which correspond to the immunoglobulins IgM, IgD, IgG, IgA and IgE (Moser and Leo, 2010). Relevant to allergy and very broadly, IgA molecules are responsible for tolerance of ingested food products, whereas IgE molecules are typically responsible for the abnormal immune response that results in allergic symptoms and responses (Nagler-Anderson, 2001).

Figure 3: Structure of immunoglobulin E



Legend. The variable region of the IgE antibody molecule enables them to bind to a range of specific antigenic peptides.

Immunoglobulin E (IgE) is the antibody responsible for immediate allergic hypersensitivity. The secretion of high levels of IgE antibody arise from the predominantly Th2 environment, rich with the cytokines IL-4, IL-5 and IL-13 (Moser and Leo, 2010). There are two phases to the immunological basis of allergic disease; the sensitisation phase and the effector phase. During the sensitisation phase, Th2 effector cells produce IL-4, which promotes class switching to the IgE heavy chain and the further production of Th2 cytokines. Allergen-specific IgE then binds to high-affinity IgE receptors - known as FcεRI- on mast cell and basophil surfaces, resulting in sensitisation. The FcεRI receptor is a receptor complex that binds the Fc section of the IgE heavy chain exon, which has a primary role in controlling the allergic response. The effector phase occurs when an individual is re-exposed to an allergen and cross-linking of the IgE FcεRI complexes on sensitised mast cells results in mast cell activation. Mast cells are present in almost all tissues and are often close to epithelial surfaces. Mast cell degranulation results in the release of pro-inflammatory mediators (including histamine, heparin, leukotrienes and cytokines), which precipitate a Type I hypersensitivity response.

1.3 Classification of hypersensitivity reactions

In 1963 Gell and Coombes described four classes of hypersensitivity based upon the mechanisms and timing involved (Gell, 1963).

Type I: immediate hypersensitivity

Type I hypersensitivity reactions are known as immediate or anaphylactic reactions. Common symptoms include one or more of the following symptoms; angioedema, urticaria, bronchoconstriction, rhinitis, conjunctivitis and anaphylactic shock. Following the binding of IgE to high-affinity receptors on the surface of mast cells and basophils, cell degranulation occurs which results in the release of preformed mediators such as histamine. This is followed by the release of newly synthesised mediators (previously known as slow reacting substances of anaphylaxis) such as leukotrienes and prostaglandins.

Type II: antibody-mediated cytotoxic reactions

Type II hypersensitivity reactions are rare cytotoxic reactions, which are antibody-mediated and are usually caused by IgG and IgM antibodies. Type II responses are associated with autoimmune diseases, adverse reactions to drugs and transplants. Reactions typically take several hours to develop. There are two mechanisms which result in tissue damage: the first results from direct action caused by neutrophils, macrophages and eosinophils and the second is due to antibody-mediated activation of the complement pathway, resulting in cell lysis. Common examples are thrombocytopenia, immunoallergic haemolytic anaemia and haemolytic disease of the newborn (Descotes and Choquet-Kastylevsky, 2001).

Type III: immune complex-mediated reactions

Immune complexes are responsible for causing tissue damage in type III reactions. Common manifestations include serum sickness and systemic lupus erythematosus. The reaction between antigen and IgM that can occur in tissue spaces results in the development of micro-precipitates around small vessels, causing cell damage. If there is an excess of antigen, soluble immune complexes develop and are deposited in blood vessel endothelium where they cause local inflammation. This culminates in complement activation, attracting macrophages, platelets and neutrophils, which further contribute to tissue damage. The primary target systems are the lungs, eyes, kidney, joints and the skin (Descotes and Choquet-Kastylevsky, 2001).

Type IV: delayed hypersensitivity

Type IV reactions primarily involve the skin, with contact dermatitis being a common manifestation. Symptoms are delayed and often occur 2-14 days after exposure, depending

upon previous exposure. Type IV reactions do not involve antibodies but occur following T cell sensitisation in conjunction with skin proteins. Reactions occur on subsequent exposure when memory T cells proliferate into effector cells (Nosbaum et al., 2009).

1.4 The development of food allergy

A food allergy is an acquired immunological reaction to a food protein, which can be IgE or non-IgE mediated. Allergy is different to other adverse reactions to foods, such as food aversion or food intolerance. IgE mediated or 'Type I food hypersensitivity', often referred to as 'true food allergy' occurs when the immune system perceives a harmless protein as potentially dangerous and responds accordingly.

The allergic response is an acquired immune response which arises due to the ability of the individual's immune system to remember and recognise a small structural component on the surface of an antigen, known as an 'epitope'. Each epitope is only able to bind with one specific IgE antibody. In allergy, the epitope is termed an 'allergen'. 30%-40% of individuals are genetically predisposed to produce specific IgE antibodies to common aeroallergens. These individuals are described as being 'atopic' or having 'atopy'. Atopic diseases include eczema, asthma, allergic rhinitis and food allergy. In predisposed atopic individuals, the response to an innocuous food allergen can be excessive, as the body mistakenly perceives the epitope as a threat. In atopic individuals, antigen presenting cells ingest the allergenic protein and present it via MHC-class II to CD4+ T-helper cells, adopting a Th2 phenotype that stimulates B cell production of IgE. Antigen-presenting cells ingest and process the allergen and display it on their surface in conjunction with MHC class II molecules. The antigen-presenting cell then migrates to the lymph nodes, where it will present its antigen to a CD4+ T-helper cell that has a complementary T cell receptor. The CD4+ T-helper cell may then meet an antigen-specific B cell that has already ingested and processed the same antigen, presenting fragments via MHC class II. The CD4+ T-helper cell will then activate the B cell, stimulating it to produce allergen-specific antibodies. IgE antibodies to food allergens are produced by plasma cells and are only able to react with the specific allergen responsible for its formation, rather like a lock and key; they are therefore known as 'specific IgE antibodies' (Sampson, 1999).

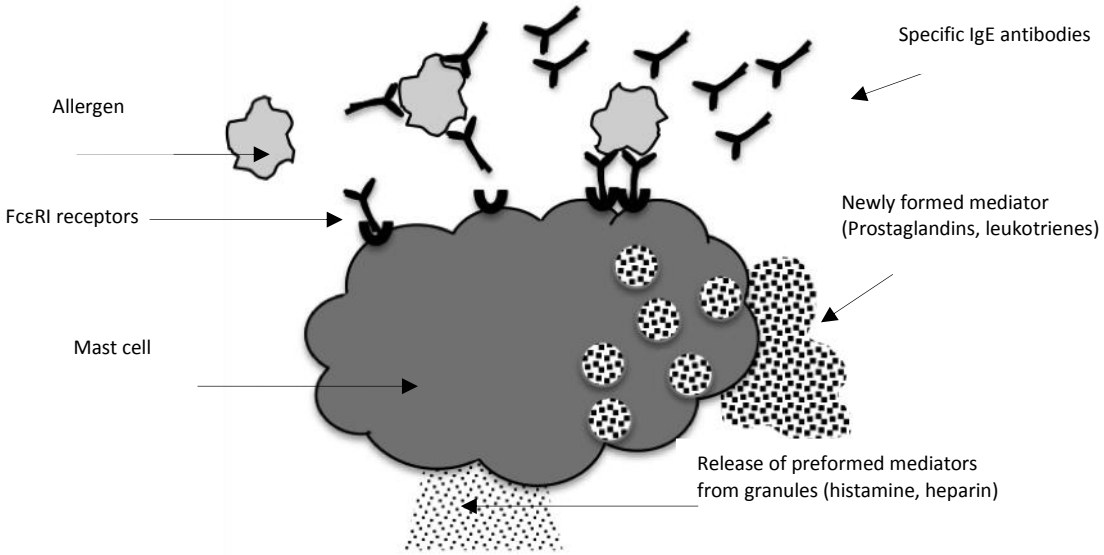
Once released, IgE antibodies bind to antigens with the aim of eliminating and neutralising their target for removal from the body. As part of this process, IgE is also bound to IgE-specific receptors (FcεRI) on the surface of mast cells on mucosal surfaces and basophils in the blood, causing sensitisation of the individual. Although no symptoms occur during the sensitisation phase, the individual becomes primed for the onset of an allergic reaction. Sensitisation is

necessary for an individual to express clinical allergy although sensitised individuals do not always express clinical reactivity. In other words, it is not possible to experience an allergic reaction to peanut without being sensitised, although some individuals may be sensitised to peanut yet be able to ingest it without reaction. It is unclear precisely why some individuals are sensitised to specific food allergens but do not experience an allergic reaction following ingestion of that food (Sampson and Ho, 1997).

Food allergens are water-soluble glycoproteins, usually less than 70 kiloDaltons (kDa) in mass, which are stable to heat, acid and proteases (Astwood, 1996, Deshpande, 1987, Sampson, 1999, Sicherer and Sampson, 2010). Allergens have several distinct molecular properties. The first is the ability to induce the immune system to produce IgE antibody and thus cause *sensitisation* of the individual. The second is the ability to trigger allergic symptoms, known as *elicitation.* Finally the allergen needs to be capable of *binding* to allergen-specific IgE.

If exposure to an allergen occurs in an allergic individual, the allergen is able to bind to and cross-link the IgE molecules and Fc receptors on the surface of the mast cells. This activates the sensitised cell, provoking degranulation. Degranulation results in the release of histamine and other pro-inflammatory chemical mediators including interleukins, leukotrienes and prostaglandins into the surrounding tissue (Figure 4). Mast cell degranulation also leads to the recruitment of additional pro-inflammatory responses (Burbank and Burks, 2015).

Figure 4: The degranulation process in a mast cell.



Legend. Degranulation of the mast cell results in the release of preformed mediators, which culminate in allergic symptoms. Adapted from Pawel Kuzniak (Kuzniak, 2006)

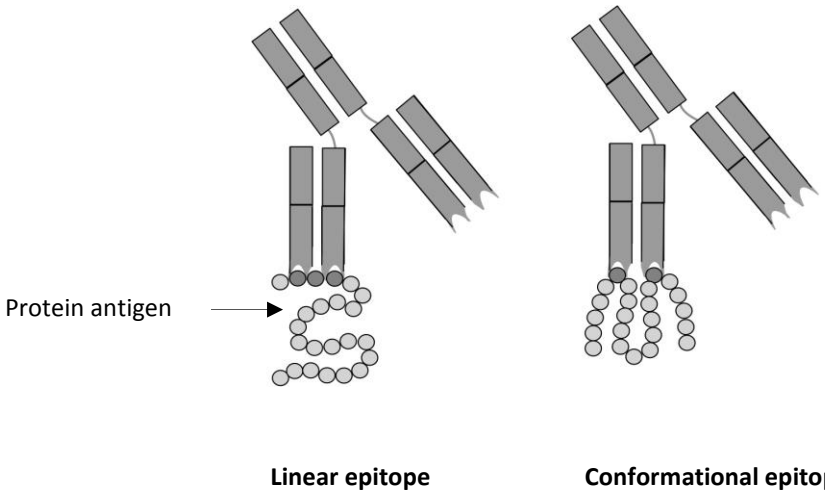
Ultimately, this culminates in unpleasant and potentially life-threatening clinical symptoms. Reactions tend to be acute in onset, often occurring immediately and usually within less than two hours. Chemical mediators affect target organs including the skin, gastrointestinal, oropharyngeal and respiratory tracts and cardiovascular systems, and reactions may be local or systemic (Iweala and Burks, 2016). Responses involving the skin causing rash or swelling are the most common and tend to be mild, whereas those involving the respiratory and cardiovascular systems are more likely to be severe (Sicherer and Sampson, 2010). The most severe form of Type I hypersensitivity is anaphylaxis, defined by the European Academy of Allergy and Clinical Immunology (EAACI) as 'a severe, life-threatening, multiple-organ hypersensitivity, often dominated by severe asthma and hypotension' (Emmett et al., 1999).

It is known that allergens have some or all of the following characteristics: stability against heat and digestive enzymes, solubility (the ability of the allergen to cross the gut mucosal barrier), and a structure allowing for surface molecule exposure (Helm, 2003). The six most common food allergens in children are milk, egg, soya, fish, wheat and peanut (Sicherer, 2002, Burks, 1998). These six foods are responsible for 90% of all reactions, although any food can trigger an allergic response.

1.5 Linear and conformational epitopes

Food allergens are water-soluble glycoproteins. A globular protein comprises a sequence of amino acids creating a protein structure that tends to be folded rather than linear. The cells of the immune system recognise 'epitopes' rather than complete antigens. An epitope, or antigenic determinant, is a sequence of amino acids (Chen, 2012). In food allergy specifically, an epitope is a localised area on the surface of the antigen that is recognised by an IgE antibody. An epitope may be either linear or conformational (3-dimensional) in structure as shown in Figure 5. A linear epitope - also known as a sequential allergenic epitope - is recognised by IgE antibodies by its contiguous sequence of five to ten amino acids. A conformational epitope has a three-dimensional shape and structure, comprised of several non-contiguous residues which are separated in the amino acid chain but which form an epitope that results from the folding of the antigen (Hansen et al., 2016, Chen et al., 2016).

Figure 5: IgE binding to linear and conformational epitopes.



Legend. The linear epitope comprises a contiguous amino acid chain whereas the conformational epitope is formed by the folding of separate amino acids. Adapted from The Immune System (Parham, 2009).

1.6 Natural history of peanut allergy

Peanut is responsible for the majority of food-induced severe allergic reactions (Macdougall et al., 2002). Type I hypersensitivity to the peanut is common, affecting 1-2% of UK school children (Tariq et al., 1996, Grundy et al., 2002, Sicherer, 2002). Peanut allergy tends to present early in life and, in more than 70% of allergic children, symptoms are present on the child's first known exposure (Sicherer, 1998). However, resolution rates are low in comparison with other foods. Approximately 50% of children with egg and milk allergy will experience resolution by two years of age (Sicherer et al., 2014, Spergel et al., 2015). Peanut allergy was previously believed to be lifelong but has subsequently been demonstrated to remit in up to

20% of primary school aged children and up to 25% of adolescents aged 10 to 18 (Lack et al., 2003b, Hourihane, 2005, Skolnick, 2001, Peters et al., 2015). It is not yet clear how to predict which children will eventually outgrow their peanut allergy. Early research suggests that resolving children may have lower initial whole peanut-specific IgE concentrations and subsequent whole peanut-specific IgE concentrations or skin prick test wheal diameters which decrease over time (Ho, 2008). These epidemiological studies demonstrate that the majority of peanut allergic children will grow up to be peanut allergic adults and therefore further research into prevention and treatment is of prime importance (Iweala and Burks, 2016).

1.7 Egg allergy and eczema - risk factors for peanut allergy

Clinicians working in allergy have to deal with an important clinical conundrum on a daily basis - what should be done with the atopic child who is already being investigated for other allergic disease, such as another food allergy or moderate to severe eczema, who is also found to be peanut sensitised? (Codreanu et al., 2011). This is a common clinical problem encountered by clinicians managing children with egg allergy. The link between egg allergy and peanut sensitisation is well established, with a significant proportion of egg allergic children being co-sensitised to peanut (Du Toit et al., 2008).

The Learning Early About Peanut allergy (LEAP) study confirms this association, reporting the presence of egg allergy to be the most important risk factor for peanut sensitisation (Du Toit et al., 2008). The LEAP study was the first study to confirm that early peanut introduction between the ages of four and ten months may prevent the development of peanut allergy in high-risk atopic children. Out of 640 infants who had never consumed peanut with severe eczema, egg allergy or both, the prevalence of peanut allergy at age 60 months was 17.2% in infants who avoided peanut and 3.2% in children who consumed 2 grams of peanut three times per week. This study provides a further important reason for clinicians to be able to differentiate between peanut allergy and tolerance in young children as easily and quickly as possible.

There were only two inclusion criteria for participation in the LEAP study; severe eczema and egg allergy. The authors propose that as egg and peanut are allergenically diverse, this association is most likely to be due to shared risk factors rather than cross-sensitisation (Du Toit, 2015). Eczema is a known risk factor for peanut allergy, with one postulated mechanism for this being the imperfect skin barrier paving the way for sensitisation to occur through the skin (Lack et al., 2003a). Lack et al (2003) proposed that sensitisation occurs through the skin via an imperfect barrier although at the time of publication the mechanism was unknown. In

2006, research identified mutations within the filaggrin gene which result in a filaggrin deficiency (Irvine and McLean, 2006). Filaggrin is a genetically regulated hydrating protein that is present within the epidermis. A genetic filaggrin deficiency exists in up to 10% of the Caucasian population and leads to a defective epithelial barrier leaving individuals prone to developing a number of inflammatory skin conditions including atopic eczema (Weidinger et al., 2008, Palmer et al., 2006). Individuals with a profilaggrin mutation may experience cutaneous dehydration causing an imperfect barrier which allows penetration by allergens (Weidinger, 2006). Subsequent studies have identified filaggrin haplo-insufficiency in a murine model to be associated with barrier impairment and percutaneous allergen sensitisation (Fallon, 2009, Oyoshi, 2009). More recently, research has been undertaken into the importance of maintaining skin integrity to prevent the development of peanut and other allergies (Brown et al., 2011, Irvine et al., 2011). Brough et al demonstrated that for children with filaggrin mutations, each log unit increase in house dust peanut protein level was associated with a six-fold increased odds of having peanut sensitisation, and more than a three-fold increase of having peanut allergy. No effect was observed in those children without filaggrin mutations (Brough et al., 2014).

Clinical practice varies with regard to testing high-risk children - particularly those with egg allergy - for peanut sensitisation. Most clinicians feel that it is best practice to actively seek out peanut sensitisation in high-risk children to avert the child from potentially having an allergic reaction at home. Others find testing for peanut sensitisation to be problematic, particularly in smaller centres, as sensitisation then requires further investigation and resources may be limited. Nicolaou reported a study in which only 22.4% of egg allergic children who were peanut-sensitised were proven to have true peanut allergy on oral provocation challenge (Nicolaou et al., 2010b).

Children with a skin prick test wheal diameter or a specific IgE antibody level above the previously published 95% positive predictive value for peanut are usually labelled as being peanut allergic, whilst those with a negative test should be encouraged to regularly consume peanut as the negative predictive value is sufficiently reliable (Burks, 1998). Rance et al reported a negative predictive value of 95% (Rance et al., 2002). However, children whose results lie within what is commonly acknowledged to be the ‘immunological grey area’ - a considerable number of children with a low or borderline skin prick or whole peanut- specific IgE test - require an oral provocation challenge to confirm or refute their peanut allergic status. If their status is not clarified, then peanut-tolerant children are at risk of developing peanut allergy by failing to include peanut in their regular diet whilst peanut-allergic children

without a clear diagnosis may not have access to appropriate medications and management advice.

It is high-risk children, whose skin prick or whole peanut-specific IgE antibody concentrations fall within this immunological grey area, for whom the greatest benefit would accrue from an improved diagnostic process, reducing the need for an oral provocation challenge. 280 oral food provocation challenges were performed at Bristol Royal Hospital for Children in 2014. 53(19%) of these were to peanut and only 4(8%) were positive, supporting the need for a highly sensitive screening test. This also suggests that referring healthcare professionals in our centre have until recently tended to err on the side of caution. Ultimately this means that many peanut-sensitised children who have never consumed peanut may not be being referred for challenges appropriately and may be being left with an incorrect diagnosis of peanut allergy. An effective allergy service could be expected to report a sizeable percentage of children who develop symptoms upon oral provocation challenge. A rate of 30-40% would be reasonable for a tertiary NHS sercice as this reflects that appropriate patients are being selected for the procedure. If all children were experiencing symptoms on oral provocation challenge, this would suggest that the centre is selecting the wrong patients for the procedure and including those who might be accurately diagnosed by skin prick or specific IgE testing and the use of positive predictive values, whilst a centre where most children do not experience any reaction are likely to be neglecting to challenge a large number of children who might actually not be allergic. For the purpose of this thesis, this group of children who have never knowingly consumed peanut will be defined as ‘peanut-naïve’ children although it is recognised that these children are likely to have had exposure to peanut in ways other than by ingestion, such as percutaneously.

1.8 Diagnosis of peanut allergy

There are considerable implications associated with a diagnosis of food allergy and establishing a child’s peanut allergic status should ideally be undertaken as early as possible with the identification of peanut-tolerant children being of prime importance. There are several reasons for this; maintaining a good quality of life, the need for allergen avoidance and provision of appropriate rescue medication, the benefit of early peanut introduction during weaning, the possibility of peanut desensitisation, the inadequacy of current screening techniques and the risks associated with an oral provocation challenge.

1.8.1 Quality of life

Peanut is a popular and widely available cheap source of protein and contamination of other food products with peanut is common (Remington et al., 2015, Brough et al., 2015). Where possible, the sooner tolerance to peanut is ascertained, the sooner a child can enjoy a normal, unrestricted diet which alleviates the social and emotional burden associated with a diagnosis of food allergy and is important for normal growth and development (Garcia-Ara et al., 2004). A diagnosis of peanut allergy often gives rise to substantial parental anxiety from fear of anaphylaxis and the constant need for vigilance (Klinnert and Robinson, 2008). Anxiety is a frequent problem for both food allergic children and their parents and can be assessed via food allergy quality of life assessments (Cummings et al., DunnGalvin et al., 2008, Flokstra-de Blok et al., 2008, Flokstra-de Blok et al., 2009, Herbert and Dahlquist, 2008, Hourihane et al., 2002, Lebovidge et al., 2009). In a nut allergic population, allergic children were found to have poorer emotional, social and psychological quality of life scores when compared with healthy normative controls (Cummings et al., 2010).

1.8.2 Allergen avoidance and appropriate medication

Allergen avoidance leads to significant dietary limitations and has implications for care at school and out of the home. As peanut allergy often provokes severe life-threatening reactions allergen avoidance is the mainstay of treatment. Of additional importance is the provision of personalised allergy management plans and emergency medications, as accidental reactions are unfortunately common (Muraro et al., 2014). Children without a confirmed diagnosis of food allergy may be excluded from school dinners, which can have financial consequences for children of low-income families, who then have to provide their children with a packed lunch themselves. This can also lead to increased segregation among school children in reception and school years one and two (Muraro et al., 2010).

1.8.3 Early introduction of peanut during weaning

There is increasing evidence to suggest that total peanut avoidance in young children may be detrimental and may lead to the development of peanut allergy (Wennergren, 2009). It is therefore important to identify children who are not peanut allergic early in life to enable parents to introduce peanut into their diets as early as possible to protect them from future allergy. Due to the strong correlation between egg allergy and peanut allergy it appears judicious to define each egg allergic child's peanut allergy status before they are accidentally exposed in the community and placed at unnecessary risk (Du Toit, 2013). The LEAP study identified that to reduce the number of children who will develop peanut allergy, peanut

should be introduced to high-risk infants (those with egg allergy or severe eczema) between the ages of 4 and 11 months of age (Du Toit, 2015). To ensure the safety of this process, ideally high-risk infants would initially be screened via skin prick testing although current national allergy service provision makes this difficult. Infants with a negative response could then introduce peanut into their diet immediately whilst those with a positive response would need to attend hospital as a day case for further clarification by way of a peanut provocation challenge. This has phenomenal health care implications both in terms of time and availability of screening services and day-case beds. There is therefore an urgent need to best identify the diagnosis of peanut allergy (or tolerance) without the need for an oral provocation challenge wherever possible.

1.8.4 Peanut desensitisation

As research into the treatment of peanut allergy continues, specifically with regard to peanut desensitisation, it is becoming increasingly important to know an individual's peanut allergic status (Blumchen et al., 2010, Jones et al., 2009, Clark et al., 2009, Kim et al., 2011). Peanut desensitisation is beginning to be rolled out and although this is currently only available privately in the UK, it is likely to be more widely available within the next few years. Pollen desensitisation is highly effective in children and young adults and it may be that early intervention may deliver the best outcome; if peanut desensitisation were to prove more effective in children than teenagers and adults, then this would present an additional case for an accurate early diagnosis. A high predictive value for early sensitisation may also provide an opportunity for a future early intervention study (Dean et al., 2007).

1.8.5 Risks associated with an oral provocation challenge

Double-blind placebo-controlled food challenges are currently the gold standard for the diagnosis of food allergy although in routine clinical practice open food provocation challenges are more usually performed as they are less resource intensive, given that they only require one hospital visit rather than two (Bock et al., 1988). Although definitive, a food provocation challenge is time consuming, expensive and carries a risk to the child. Positive oral food provocation challenges may provoke acute allergic reactions with potentially life-threatening anaphylaxis (Nowak-Wegrzyn et al., 2009). Consequently it is far from being an ideal test as there is both a risk to the child and a significant cost implication. Reduction of the need for an oral provocation challenge would also have an important effect upon service delivery. Waiting lists can be long, staff-to-patient ratio requirements are high and the procedure is costly. The cost to the NHS of an oral provocation challenge at Bristol Royal Hospital for Children lies between £453 and £1118, depending upon challenge outcome and the child's co-morbidities.

Children who pass incur a charge to Primary Care of £453, those who fail incur a cost of £563 and for those with a co-existent diagnosis of asthma, the charge is £1118. Establishing a simple, reliable test to reduce the number of oral provocation challenges referrals would therefore bring considerable health economics benefits. As the burden of allergy increases, the waiting lists for such challenge tests grow longer and the need for improved diagnostics becomes increasingly important.

1.8.6 Inadequacy of current screening techniques

A diagnostic screening test for the diagnosis of peanut allergy in high-risk, peanut-naïve infants would be extremely useful in clinical practice. Food allergy in children is often parentally diagnosed, with the incidence of parentally-perceived food allergy being significantly higher than physician-diagnosed food allergy (Eggesbo et al., 1999, Eggesbo et al., 2001, Pyrhonen et al., 2009). Several studies have attempted to identify diagnostic markers that can predict the likelihood of an allergic reaction during an oral peanut provocation challenge (frequently referred to as a positive oral provocation challenge) and thereby lessen the need for the test. These studies have investigated the clinical utility of a number of variables, including both clinical symptoms and immunological markers such as specific IgE and skin prick test values (Hill et al., 2001, Sporik et al., 2000, Hill et al., 2004, Savage et al., 2007, Boyano-Martinez et al., 2002). Only one in five children with measurable whole peanut-specific IgE will have clinical reactivity on exposure to peanut.

The opportunity to establish safe, reliable in vitro testing for food allergy has been a focus since the late 1990s when early work on the development and clinical utility of positive predictive values for peanut allergy was published (Sampson and Ho, 1997). Much focus has been placed upon the possible role of positive predictive values in the diagnosis of peanut allergy. The positive predictive value (PPV) is the proportion of patients with a positive test result who prove to be allergic on oral provocation challenge. It reflects the presence of a positive screening test successfully identifying the underlying condition being tested for. Measures of diagnostic performance also consider sensitivity and specificity (type I and type II errors). Sensitivity measures the proportion of children whose peanut allergy is correctly diagnosed by their positive test result whilst specificity measures the proportion of negative results that are correctly identified by the test. An allergen-specific IgE positive predictive value with a threshold of 95% is a cut-off value for allergy-specific IgE that is exhibited by 95% of children who undergo an oral provocation challenge and subsequently have a confirmed allergic reaction. The validity and clinical utility of previously published positive predictive values are examined later in this chapter. Published positive predictive values vary between

studies and depend upon the study population, as the positive predictive value is dependent upon disease prevalence within a given population. The diagnosis of peanut allergy in a peanut-naïve sensitised individual therefore currently continues to be dependent upon an oral food provocation challenge as existing in vivo and in vitro testing is insufficiently reliable to make an accurate diagnosis in a large number of peanut-sensitised children.

1.9 Assessment of food allergy status

The current diagnostic approach to any food allergy begins with the taking of an allergy-focussed clinical history of any symptoms that may be indicative of a Type 1 hypersensitivity response. Children may be sensitised to peanut yet remain clinically asymptomatic without expressing symptoms of clinical food allergy on ingestion. A diagnosis should therefore be based on a positive clinical history in conjunction with the presence of food-specific IgE antibodies. The presence of food-specific IgE antibody levels can be determined either in vivo (by skin prick testing) and/or in vitro (by measuring food-specific IgE in serum). Component testing (i.e., IgE that is specific for sub-components of foods, such as specific proteins within a peanut) is currently only routinely available via specialist allergy services and not routinely used in smaller hospitals without an in-house immunology laboratory. In the Bristol region, access to specific-IgE testing is controlled by the laboratory manager and GPs are forbidden from requesting certain tests, including component testing. This is to restrict costly, unnecessary and inappropriate testing within an environment in which the specialist allergy knowledge required to interpret such tests is lacking.

1.10 In vitro tests for peanut allergy

Laboratory testing for whole peanut-specific IgE testing is a standard routine investigation recognised globally. As discussed above, work has been conducted on the development and clinical utility of positive predictive values for peanut allergy. Predictive cut-off values reported in the literature are often lower in infants and small children and increase with age (Benhamou et al., 2009). Published 95% positive predictive values for a clinical reaction to peanut are depicted in Table 1, highlighting the substantial differences in whole peanut-specific IgE positive predictive values between published studies (Peters et al., 2013, Wainstein et al., 2007, Roberts and Lack, 2005, Rance, 2002).

Table 1: Summary of published positive predictive whole peanut-specific IgE cut-off values				
Author	**Date**	**No. of children**	**Cut-off value**	**%PPV**
Peters et al (2003)	2013	438	≥34 kUA/l	95%
Wainstein et al (2007)	2007	85	>10 kUA/l	100%
Roberts et al (2005)	2005	161	≥15 kUA/l	95%
Rance et al (2002)	2002	363	>57 kUA/l	100%
Sampson and Ho (1997)	1997	196	≥15 kUA/l	95%

Legend: Positive predictive values vary widely between studies.

The positive predictive values of 15 kUA/L (kilo-units of antibody per liter) or higher identified by Sampson is the most frequently used value in UK clinical practice and the value most usually referenced in peanut allergy research papers (Sampson and Ho, 1997). Explanations for the differences seen in other studies may include the age of the study population, varying selection criteria and varying standards for defining an oral provocation challenge outcome (as either a pass or fail). The inclusion of subjective or very mild symptoms as positive has clear implications for the diagnostic values. Study populations also vary, and Roberts and Lack propose that published values for peanut skin prick testing or whole peanut-specific IgE concentrations in children from a tertiary allergy clinic cannot be generalised for use in other community-based populations (Roberts, 2005). Benhamou et al suggested that for future studies, well-characterised clinical phenotypes and standardized challenge protocols which include food preparation might elicit more valuable predictive information (Benhamou et al., 2009).

1.11 In vivo tests for peanut allergy

Skin prick testing is the process whereby a minute quantity of allergen is introduced into the epidermis where it is able to interact with specific IgE bound to mast cells (Dreborg, 2001). The diameter of the resulting wheal produced in response is measured with a ruler. This is an internationally popular diagnostic tool of choice in paediatric allergy clinics as results are immediate and the test is cheap and simple to perform. As with allergen-specific IgE tests whereby IgE is measured in serum samples, there are limited studies available comparing skin prick test wheal diameters with the results of an oral provocation challenge. Of the few studies that have been published, the results vary substantially (Sporik et al., 2000, Hill and Hosking, 2004, Peters et al., 2013, Wainstein et al., 2007, Nolan et al., 2007, Roberts, 2005). Published positive predictive skin prick test wheal diameters for whole peanut are shown in Table 2.

Table 2: Summary of published positive predictive cut-off values for peanut skin prick testing

Author	Date	Cut-off value
Peters et al (2013)	2013	≥15mm
Nolan et al (2007)	2007	>7mm
Wainstein et al (2007)	2007	≥15mm
Roberts et al (2005)	2005	≥8mm
Hill et al (2004)	2004	≥8mm
Sporik et al (2000)	2000	≥8mm

Legend: Positive predictive values for skin prick tests vary widely between studies.

The most frequently quoted skin prick test cut-off value for predicting a clinical reaction to peanut in clinical practice is 8mm, although approximately 43% of children with a skin prick test wheal diameter below this cut-off value will still experience a reaction during an oral provocation challenge. Peters' systematic review identified two groups of values and reported that the higher values were elicited by prick-to-prick testing rather than the conventional standardised extracts (Peters et al., 2013). Some studies did not differentiate between sensitised and proven allergic children, which may have led to an inaccurate positive predictive value calculation. For example, one study assessed the positive predictive values among all children with a positive skin prick test to peanut within a tertiary allergy clinic, regardless of whether they had any history of a clinical reaction (Wainstein et al., 2007).

1.12 Component-resolved diagnostics for peanut allergy

Traditionally, the investigation of food allergy has been by either skin prick testing or measuring whole peanut-specific IgE as described above. More recently, due to advances in diagnostic testing procedures, it has become possible to test for smaller and more specific allergenic parts of food allergens containing different epitopes; this is known as *component-resolved diagnostics (CRD).* Component-resolved diagnostics is a method of identifying the allergenic sensitisation profile of patient at a pure molecular level by using recombinant allergenic molecules instead of allergen extracts (van Veen et al., 2016). Recombinant allergens are biotechnologically produced allergenic molecules that have been identified from an original allergen extract. They have IgE antibody binding capacity comparable to that of the natural allergen and are similar to the original protein with regards to structural features and immunological properties. Component testing evaluates the binding of IgE to specific allergenic proteins known as *components* (Valenta et al., 2007). Component testing is deemed superior to whole allergen testing as it is essentially cleaner; testing based on natural allergen

extracts is composed of ill-defined mixtures of major allergens, cross-reactive components and non-allergenic material making it more difficult to identify the disease-eliciting allergen (Bousquet et al., 1998).

Theoretically, it may be possible to use component-resolved diagnostics to distinguish between individuals with a genuine peanut allergy and those who have positive whole peanut-specific IgE or positive skin prick tests to peanut due to cross-reactivity. As such, they have the potential to make it easier to differentiate between peanut allergic and tolerant children without the need to subject them to an oral provocation challenge. As described below, some peanut allergen components are associated with milder symptoms, others cross-react with pollen allergens, whilst the seed storage protein components have a more sinister profile. Component testing is currently only routinely available via allergenic component-specific IgE measured in serum although skin prick reagents may be made available in the future.

1.13 Peanut allergens

The peanut is the fruit of the groundnut plant, which splits into two halves to reveal the embryonic plant (plumule). The main body of the peanut, known as the cotyledon, stores those nutrients required to support the germinating plant. A cotyledon is the primary or seed leaf in the embryo of higher plants. The protein element of the cotyledon is the most allergenic part of the peanut and contains the seed storage proteins important to the developing plant. Seed storage proteins are highly allergenic, being both heat and digestion stable and not easily altered from their most allergenic form (Lehmann et al., 2006). The more stable an epitope, the higher the risk of severe allergic reactions (Flinterman et al., 2008).

The terminology of peanut allergenic components relates to the Latin genus and species name of the plant (‘Ara h’ for ‘Arachis hypogaea’), with each discrete component being numbered sequentially. Thirteen peanut components have been identified to date; the most immunologically important are considered to be Ara h 1, 2, 3, 6, 8 and 9 (Becker and Jappe, 2014).

1.13.1 Seed storage proteins

Seed storage proteins are digested during germination. Proteins from legumes belong to the globulin family of seed storage proteins and are categorised as either legumins (known as 11S globulin) or vicilins (known as 7S globulin) (Shewry et al., 1995, Breiteneder and Mills, 2005). Globulins are a family of globular proteins that have higher molecular weights than albumins. Each globulin has its own particular shape. Together, Ara h 1, 2 and 3 represent more than 30%

of the total peanut protein content (Chassaigne, 2007). Storage proteins remain unaltered by heat or digestion and are therefore able to cross the gastrointestinal mucosal intact.

Ara h 1 is a glycosylated seed storage protein belonging to the vicilin (7S) family of seed storage proteins and a member of the cupin superfamily, in addition to the 11S seed storage protein, Ara h 3, which is a peanut glycinin belonging to the legumin (11S) family (Lehmann et al., 2006, Koppelman et al., 2003, Koppelman et al., 2001, van Boxtel et al., 2006). Ara h 1 comprises 12 to 16% of the total peanut protein and to date 23 linear binding epitopes have been identified (Burks, 1997).

Ara h 2 is a conglutin seed storage protein related to the 2S albumin family, along with Ara h 6 and Ara h 7. There is reported to be significant sequence homology between these three components (Lange et al., 2014). Ara h 2 is a 17 kDa glycoprotein comprising between 5.9-6.3% of the total peanut protein. Like Ara h 2, Ara h 6 is a 2S albumin, sharing several epitopes with Ara h 2 and although it rarely exists in isolation it can provoke systemic allergic reactions (Chen et al., 2013, Koppelman et al., 2005, Suhr et al., 2004). The exact role for Ara h 2 in peanut allergy has not yet been well defined. Ara h 2-specific IgE sensitivity does not appear to have the same geographical distribution as Ara h 1; Ara h 1 sensitivity is recognised in almost all North American allergic patients, but in only up to 35% of European allergic populations (Burks, 1997). Ara h 2 is known to exist in two isoforms: Ara h 2.0101 and Ara h 2.0201 - isoforms being different forms of the same protein structure (Hales, 2004).

1.13.2 Profilin

Profilins are proteins found in plant species and plant foods, which have extensive cross-reactivity. Profilin sensitisation is common among pollen-sensitised children with Ara h 5, a pollen profilin homologue, being recognised in approximately 10% of peanut allergic children (Kleber-Janke, 1999). It is primarily associated with local reactions and many sensitised individuals will not react at all when challenged (Pele, 2010).

1.13.3 Bet v 1 homologue pathogenesis-related protein (PR-10)

A Bet v 1 homologue is a protein that is similar in structure to the birch pollen protein Bet v 1 (*Betula verrucosa),* the primary allergen in Silver Birch pollen. Bet v homologues are classified as pathogenesis-related proteins, most commonly referred to as PR-10 proteins, and are found in plant foods and in tree pollen. They are part of a plant's defence system against ubiquitous pathogens and are activated by infection (Fernandes et al., 2013). There are seventeen families of PR proteins, with classification being dependent upon structure. Bet v homologues fall into

the family PR-10 (Fernandes et al., 2013, He, 2014). Ara h 8 is a minor allergen, which cross-reacts with the birch pollen allergen Bet v 1 as a consequence of its similar protein fold structure, and sensitisation depends largely upon pollen exposure (Lange et al., 2014). This Bet v 1 homologue PR-10 protein is associated with mild to moderate symptoms in individuals with concurrent birch-pollen induced hay fever (Asarnoj et al., 2012a, Hurlburt et al., 2013). Ara h 8 is a labile protein, unstable against digestion and which has low stability when roasted (Mittag et al., 2004). The Bet v 1 homologue, Ara h 8, appears to provoke symptoms of the pollen fruit syndrome in individuals sensitised to the birch pollen Bet v 1, whilst Ara h 9-specific IgE is most common in individuals exposed to pollens from the Fagales plant order (Ebisawa, 2012). The minor peanut antigens may be less likely to provoke a severe anaphylactic reaction and more commonly associated with oral symptoms. This was demonstrated in a Swedish population study of adolescents and adults which identified sensitization to Ara h 1-3 as a risk factor for systemic reactions whilst individuals sensitised to either Ara h 8 or 9 did not report severe symptoms (Kim and Nowak-Wegrzyn, 2011). A study of peanut component-IgE recognition patterns in 11 European countries reported that peanut-tolerant subjects were frequently sensitised to Ara h 8 or 9 but not to the seed storage proteins (Ballmer-Weber, 2015). However, case reports of severe reactions to Ara h 8 sensitised individuals have been published (Glaumann et al., 2013).

1.13.4 Non-specific Lipid transfer protein (Ara h 9)

Lipid transfer proteins are proteins that are present in plant foods and in tree and weed pollens, being most commonly found in fruits, vegetables and nuts. They are fairly stable structures and can result in systemic reactions but are regarded as secondary food allergens. Ara h 9 is a lipid transfer protein with some cross-reactivity to profilin and is predominantly found in Mediterranean individuals (Asero, 2002, Krause et al., 2009). Ara h 9 sensitised individuals are often co-sensitised to components Ara h 1-3 which may explain why these individuals are also frequently prone to severe systemic reactions, although Ara h 9 also possesses some thermal and digestive stability (Moverare et al., 2011, Lauer et al., 2009, Blom et al., 2013).

1.13.5 Oleosins

Oleosins are low molecular weight structural plant proteins that are involved in the formation of oil bodies; they are potential allergens in legumes, tree nuts and oils. It has been postulated that they may be responsible for allergic reactions to peanut oil and potentially cross-react with soya although their role in peanut allergy is likely to only affect a small number of peanut allergic individuals (Pons et al., 2002, Lange et al., 2014).

1.13.6 Defensins

Defensins such as Ara h 12 and 13 are low molecular weight allergens and have been recently associated with severe clinical allergic reactions to peanut (Petersen et al., 2015). The primary molecular characteristics of the peanut components are shown in Table 3 (Matsuo et al., 2015, Pele, 2010).

Table 3: Molecular characteristics of the major peanut allergens

Nomenclature	Protein family	Family type	Characteristic	Probable implications for clinicians?
Ara h 1	7S Vicilin (Conarachin)	Seed storage protein	Heat stable Degradable by gastric digestion but some allergenicity retained Associated with severe reactions	Associated with significant reactions
Ara h 2	2S Albumin (Conglutin)	Seed storage protein	Heat & digestion stable Associated with severe reactions	Highly allergenic Most important predictor of peanut allergy
Ara h 3.01	11S Globulin	Cupin (legumin) Seed storage protein	Heat stable Degradable by gastric digestion but some allergenicity retained Associated with severe reactions	Associated with significant reactions
Ara h 3.02 (previously Ara h 4)	Glycinin	Cupin (legumin) Seed storage protein	Heat & digestion stable Associated with severe reactions	Associated with significant reactions
Ara h 5	Profilin	Pollen-associated allergen	Highly cross-reactive and present in most plants	Seldom associated with clinical symptoms
Ara h 6	2S Albumins	Conglutin homologue protein	Heat & digestion stable Trypsin inhibitor	Substantial cross-reactivity with Ara h 2, but exact role not well clarified
Ara h 7	2S Albumins	Conglutin homologue protein	Heat & digestion stable	One of the least studied allergens
Ara h 8	Pathogenesis-related protein family (PR-10) (Bet v 1 homologue)	Pollen-associated allergen	Heat labile protein Cross reacts with birch pollen Associated with fruit & vegetable reactions (pollen fruit syndrome) in North Europe	Usually associated with local symptoms
Ara h 9	Non-specific Lipid Transfer Protein (nsLTP)	Plant panallergens	Heat & digestion stable Associated with fruit & vegetable reactions (oral allergy syndrome – OAS) in South Europe	Possible association with systemic, severe reactions in addition to OAS. Primarily in Mediterranean countries.
Ara h 10/11	Oleosin	Structural protein	Low molecular weight protein involved in oil formation	May be associated with peanut oil reactions
Ara h 12/13	Plant defensins	Plant proteins	Low molecular weight proteins	Recent association with severe reactions

Legend. Identification of an individual's peanut sensitisation profile may assist in assessment of the risk of an individual experiencing a severe systemic reaction.

1.14 Allergenicity and denaturation of peanut allergens

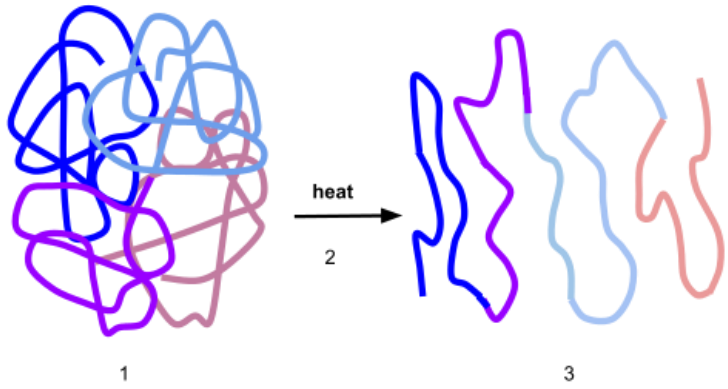
Digestive enzymes in the gastrointestinal tract normally degrade food constituents during passage between the mouth and the small intestine. In order to cause a reaction, allergenic proteins, or fragments of proteins, are absorbed by the gut mucosa, processed by dendritic cells and then presented to the immune system. The stability of an allergen is an important factor as the longer the significant component of the allergenic protein remains intact, the more able it is to trigger an allergic reaction (Astwood, 1996). The more stable a protein, the

easier it is for it to cross the gastrointestinal mucosa and provoke systemic reactions (Moreno, 2007). Most allergens enter the body via the mucosal surfaces. The intestinal epithelial layer has a complex dual role of facilitating the digestive absorption of nutrients whilst preventing access to potential antigens (allergens) and pathogens, aided by 'tight junctions' which restrict access to all but the smallest molecules (<2kDa). Specialist epithelial cells, known as 'M cells', transport microbes (and allergens) to antigen presenting cells within the gut-associated lymphoid tissue (GALT). Secretory IgA also has a role in excluding access to the gut epithelium from antigenic proteins and may have a role in the induction of oral tolerance (Nagler-Anderson, 2001).

Analysis of highly allergenic food substances has identified biochemical characteristics which are shared by many food allergens (Stanley, 1999). Many food allergens contain intramolecular disulfide bonds which are of paramount importance to their allergenicity (Taylor, 1996). Denaturation is the process by which these disulfide bonds are either altered or destroyed. The destruction of these disulfide bonds disrupts the native conformation and changes the tertiary structure of the protein (Figure 6). Following protein denaturation the protein shape is modified with the consequence that the IgE binding site no longer exists (Siskiyous, 2010). Once a protein has been denatured it frequently loses its allergenic potential, as IgE binding to the specific epitope is no longer possible.

The majority of epitopes are conformational. Conformational epitopes are generally more labile and easily destroyed by heat than linear epitopes. Following denaturation, the amino acid sequences of linear epitopes may sometimes still be recognised by the antibody, and therefore retain some of their binding capacity. Conformational epitopes are generally unable to bind to the antibody after denaturation as unfolding destroys the shape of the amino acid sequence. A lack of IgE recognising linear epitopes has been postulated as the likely mechanism by which some children experience resolution of peanut allergy (Hourihane, 2005). Conformational models of Ara h 2 and Ara h 6 demonstrate almost identical protein structures (Lehmann et al., 2006, Flinterman et al., 2007).

Figure 6: The process of globular denaturation



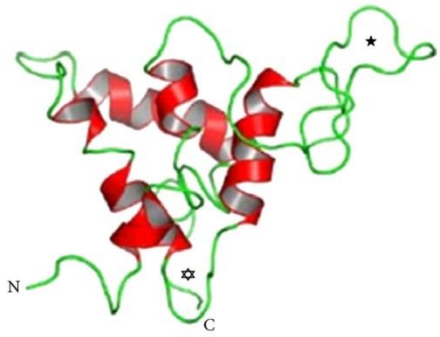
Legend. The process of denaturation alters the shape of the proteins, which subsequently lose their allergenic potential due to the inability to bind to the specific epitope. Above, the original protein (1) is altered by heat (2) to assume an altered form after denaturation (3) (***Scurran15, 2015)***.

Common denaturation parameters include ranges of pH and temperature. Individual peanut proteins have differing denaturation parameters; for example, the allergenicity of Ara h 1 has been demonstrated to be heat-stable although conformational changes may be induced by heat treatment (Koppelman, 1999). Both Ara h 1 and Ara h 3 have been demonstrated to be more easily hydrolysed by the digestive enzyme pepsin, whilst Ara h 2 and Ara h 6 remain digestion stable (Koppelman et al., 2010). However, although gastric digestion results in rapid degradation of Ara h 1 and Ara h 3 into small fragments, it retains some T cell stimulatory and IgE-binding properties although in contrast, Ara h 2 is far more stable (Eiwegger et al., 2006, Koppelman et al., 2010). Ara h 2 has been shown to be resistant to acidic environments and consequently to digestion by gastrointestinal tract enzymes (Astwood, 1996).

1.15 Ara h 2 and persistent peanut allergy

Recent research has identified that the measurement of Ara h 2-specific IgE may be of considerable value in distinguishing between children with true clinical allergy and those who are merely sensitised. The three dimensional structure of Ara h 2 shown in Figure 7 comprises a five-helix bundle stabilised by four disulfide bonds and is similar in structure to many amylase and trypsin inhibitors (Mueller et al., 2011). The disulfide bonds are of paramount importance to the ability of Ara h 2 to resist denaturation and subsequently to its ultimate stability as described above (Sen, 2002). Mapping of the Ara h 2 epitope-binding sites has identified ten epitopes (Barre et al., 2008). Ara h 2 has been identified as being a more potent allergen than either Ara h 1 or 3 (Palmer, 2002, Koppelman, 2004).

Figure 7: Ribbon diagram of the peanut component Ara h 2



Legend. The glycoprotein, Ara h 2, is a three-dimensional conformation comprising a five-helix bundle stabilised by four disulfide bridges which accounts for 5.9-6.3% of the total peanut protein. To date, ten epitopes have been mapped on the molecular surface (Barre, 2005).

As Ara h 2 retains it allergenic properties despite digestion and extensive heating, the measurement of specific IgE antibodies to Ara h 2 has been suggested to be of value in the identification of peanut-sensitised children who are allergic to peanut, eliminating unnecessary challenges and risk (Klemans et al., 2015, Klemans, 2013, Dang et al., 2012). More than 95% of American peanut-allergic individuals have positive Ara h 2-specific IgE concentrations (Koppelman et al., 2001, Palmer et al., 2005).

A literature review to identify relevant articles related to Ara h 2-specific IgE testing in the diagnosis of peanut allergy is presented in Chapter 2. An increasing number of medical practitioners working in the field of paediatric allergy are beginning to consider the measurement of Ara h 2-specific IgE concentrations as a useful adjunct to their existing practice and clinical decision-making. The literature review aims to establish what is currently known about the clinical utility of Ara h 2-specific IgE testing in peanut allergy diagnosis and to ascertain where knowledge is lacking and further research may be required.

Chapter 2

Literature review

Edwin Rosskam. Peanut vendor outside the White House. Washington, D.C. 1940.
Repository: Library of Congress Prints and Photographs Division Washington, DC 20540 USA http://hdl.loc.gov/loc.pnp/pp.print Rights Advisory: No known restrictions.

CHAPTER 2

LITERATURE REVIEW

2.1 Search methods for Identification of relevant literature related to peanut and Ara h 2

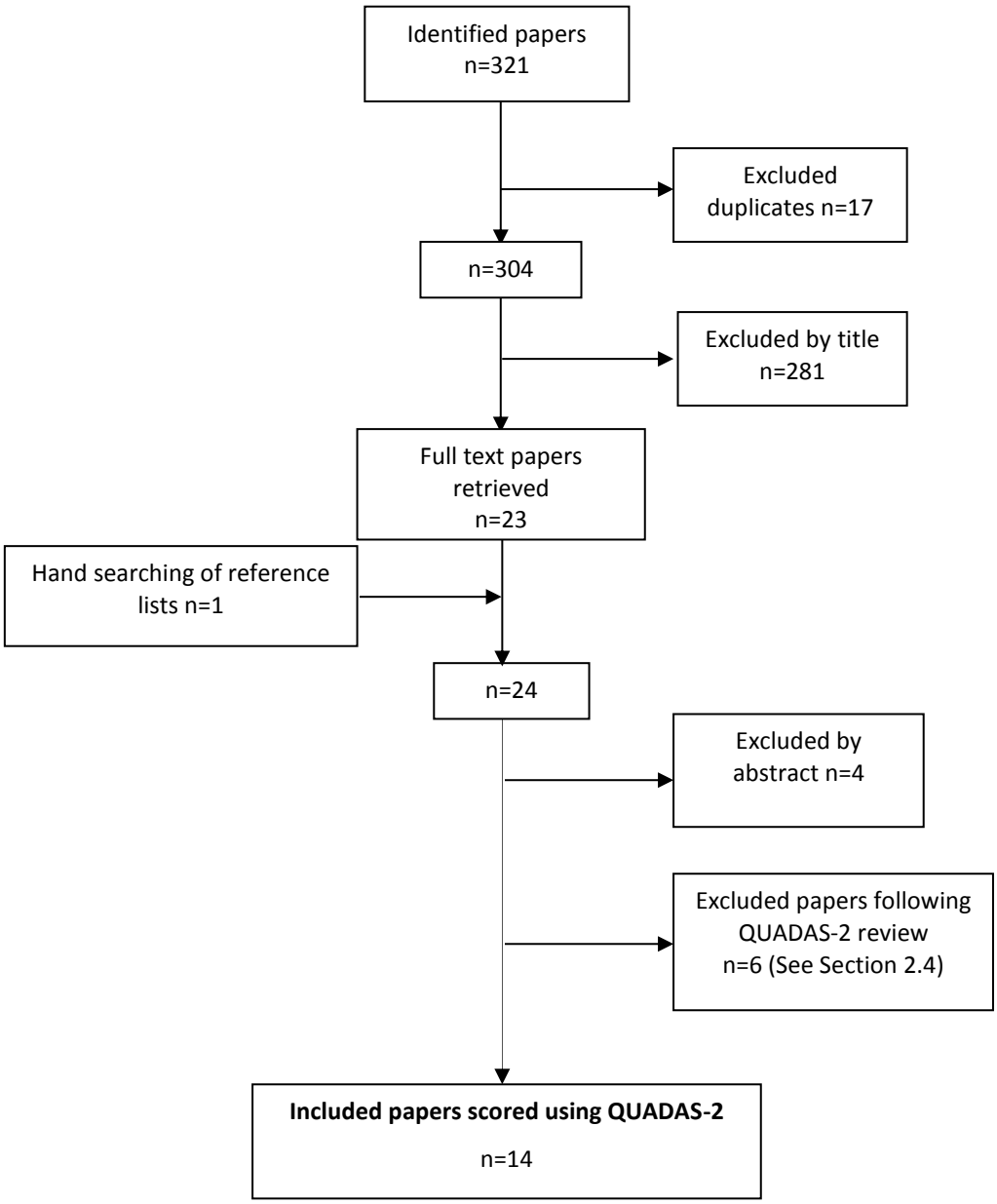
This review searched the PubMed Databases for articles published between 2010 to June 2016. This is because component-resolved diagnostic testing is a relatively new concept and searching earlier databases was unlikely to have identified relevant papers. The initial literature review was conducted on 19.05.2015 but was repeated on 27.06.2016 following completion of the study after several additional relevant articles were published. This later review identified an additional 9 articles of potential interest. The search terms used in this literature review are shown in Table 4. No language restrictions were made to maximize international coverage although search was restricted to journal articles only, with presentation abstracts being excluded. Abstracts were searched by hand for relevance and the full text of relevant papers was acquired and read. Salient points of the abstract were noted and important references obtained. A search of the term 'Ara h 2' (search 7) identified 158 titles and abstracts in the PubMed database published between 2010 and June 2016; 21 of these were relevant. After the exclusion of duplicate papers, a second search using the terms 'peanut allergy (search 3) and diagnosis (search 6) identified only 1 additional paper. A further additional paper was obtained from the hand-search of reference lists. Other search terms were employed as outlined below but no additional papers were found.

Table 4: PubMed Search History (27.06.2016)

Search No:	Search History	Results
1	Hypersensitivity	31520
2	Peanut hypersensitivity	127
3	Peanut allergy	936
4	Screening	182676
5	Component resolved diagnostics	50
6	Diagnosis	475013
7	Ara h 2	158 (21)
8	3 and 5	13(0)
10	3 and 6	150 (1)

The breakdown of the search history is depicted in Figure 8. After exclusion of duplicate articles and those deemed irrelevant by title, 23 papers were obtained. Hand searching of reference lists identified 1 additional relevant paper. After the papers had been read, they were scored using the QUADAS-2 (Quality Assessment Diagnostic Accuracy Study) scoring system, a tool for assessing the quality of diagnostic accuracy tests (Whiting, 2011). The QUADAS tool has been utilised in several systematic reviews and recommended by the National Institute for Clinical Excellence (NICE).

Figure 8: PubMed Search History (27.06.2016)



2.1.1 Papers excluded by abstract

4 papers were excluded by abstract. Tuano et al published an instructive article on component-resolved diagnostic methodology, Bernard et al investigated the relative contributions and allergenicity of linear and conformational epitopes of Ara h 2, Kukkonen et al aimed to utilise Ara h 2-specific IgE to distinguish between mild and moderate-to-severe peanut allergy in a birch-pollen endemic area and Namork looked at age-related differences in peanut-sensitisation patterns in a cohort of patients voluntarily reported to the Norweigan Food Allergy Register with no reference to oral provocation challenges (Namork and Stensby, 2015, Kukkonen et al., 2015, Bernard et al., 2015, Tuano and Davis, 2015).

2.2 Literature review methods

A systematic literature review was performed of all relevant identified papers describing the clinical utility of measuring Ara h 2-specific IgE for the diagnosis of peanut allergy in children. The quality of the included studies was assessed using the QUADAS-2 checklist (see Table 5). QUADAS-2 is a validated tool designed to evaluate the risk of bias and applicability of primary diagnostic accuracy studies through four phases. The first phase produces a review question based on the patients studied, the index test and the reference standard and reference condition. This is similar to the PICO process, used as the simplistic basis for this review. The PICO process is used in evidence-based practice to examine a clinical care-related question. The acronym stands for: P – patient or study population, I – intervention, C – comparison and O – outcome (Huang et al., 2006). Studies were included if they focused on children with either suspected peanut allergy or peanut sensitisation (based on skin prick or specific IgE testing) as these are the study populations examined within this thesis. There were no studies found specifically examining the clinical utility of Ara h 2-specific IgE concentrations for the diagnosis of peanut allergy in egg allergic children.

The phase one PICO question was utilised as follows:

Patients:	Children with suspected peanut allergy
Intervention (Index Test):	Ara h 2-specific IgE measured
Comparison Test:	Oral Provocation Challenge
Outcome:	Confirmed peanut allergic status

Phase two examines the construction of signalling questions to be used to assess study validity, phase three aims to review the flow diagram for the primary study and phase four is designed to apply judgments regarding study bias and applicability. This review focuses

primarily upon phases one and four. Following the literature review, eligibility criteria for selected studies were developed and are listed in Table 5.

Table 5: Study inclusion and exclusion criteria

Inclusion criteria:	Exclusion criteria:
Articles only	Non-English language
Suspected peanut allergy	Published pre- 2010
Index test performed	Other Index Test
OPC in 20% of study population	Systematic review or review article
Paediatric population	No paediatric subjects

The QUADAS-2 scoring assessment tool depicted in Table 6 is relevant to phase four of the QUADAS-2 process; papers are assessed for risk of bias (sub-domain A) and concerns regarding test applicability (sub-domain B) through focusing on 4 key domains: patient selection; use of the index test; use of the reference standard, and flow and timing.

Table 6: QUADAS-2 Scoring Assessment Tool

Risk of Bias Domain	
Patient selection	☹ Patients were not randomly or consecutively selected; case-control design was used; non-comparable cohorts were used
Index test	☹ Cut-off value not specified in the paper
Reference standard	☹ An open challenge rather than a DBPCFC was performed on more than 50% of study population
Flow and timing	☹ The reference standard was not performed in >5% the study population
Applicability Concern Domain	
Patient selection	☹ Non-secondary or tertiary care centre; diagnosis based on sensitization regardless of clinical history
Index test	☹ Non standardized / commercially available test performed
Reference standard	☹ Weak criteria for categorization of OPC result, which may allow for misclassification of diagnosis

☺ Low Risk ☹ High Risk ? Unclear Risk

Studies deemed eligible and subjected to a QUADAS-2 quality assessment are summarised in Table 7 and Figure 9, formatted using recommended QUADAS-2 resources (http://www.bristol.ac.uk/social-community-medicine/projects/quadas/quadas-2/) (QUADAS-2, 2016).

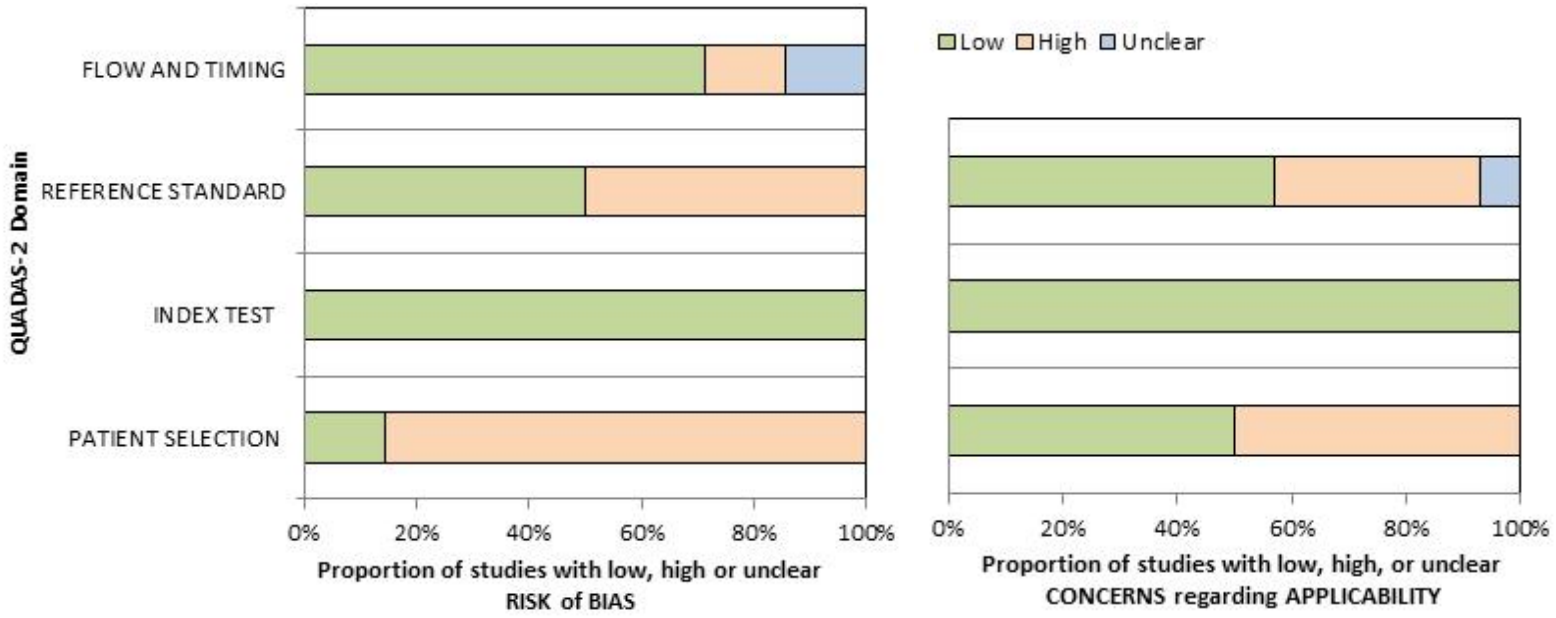
Figure 9 clearly summarises the studies assessed in Table 7. It is clearly evident that the domain with the highest risk of bias was the patient selection domain, followed by the reference standard domain. Risk of bias was lowest in the index test and flow and timing domains. 14 included studies are synopsised individually in Section 2.3 using the QUADAS-2 scoring assessment tool outlined above in Table 6. Six selected studies which did not fulfil the eligibility criteria but which still report findings of interest regarding the diagnostic utility of Ara h 2-specific IgE concentrations are described separately at the end of the chapter in Section 2.4.

Table 7: Quality assessment of eligible articles using QUADAS-2

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Kim, 2016	☹	☺	☹	☺	☹	☺	☺
van Veen, 2016	☺	☺	☺	☺	☺	☺	☺
Martinet, 2016	☹	☺	☺	☹	☹	☺	☺
Beyer, 2015	☺	☺	☺	☺	☺	☺	☺
Ebisawa, 2015	☹	☺	☺	?	☹	☺	?
Leo, 2015	☹	☺	☹	?	☹	☺	☹
Eller, 2013	☹	☺	☹	☺	☹	☺	☺
Klemans, 2013	☹	☺	☺	☺	☹	☺	☺
Lieberman, 2013	☹	☺	☺	☺	☹	☺	☺
Lopes de Oliveira, 2013	☹	☺	☺	☺	☹	☺	☺
Surtannon, 2013	☹	☺	☹	☹	☹	☺	☹
Dang, 2012	☹	☺	☹	☺	☹	☺	☹
Ebisawa, 2012	☹	☺	☹	☺	☹	☺	☹
Nicolaou, 2010	☹	☺	☹	☺	☹	☺	☹

Legend: ☺**Low Risk** ☹**High Risk** **?** **Unclear Risk** ***Most studies had bias in the patient selection domain and many within the reference and flow and timing domains. No study introduced bias within the index test domain.***

Figure 9: Proportion of studies with low or high risk of bias and applicability



2.3 The diagnostic utility of detecting Ara h 2-specific IgE: Selected articles identified from the literature search

Peanut comprises several discrete allergenic proteins. The development of component-resolved diagnostics for peanut, where component-specific IgE concentration levels are measured, is anticipated to be a valuable tool in the diagnosis of peanut allergy. Ara h 2 has been proposed as the immunodominant allergen in peanut allergy and subsequently a potential superior diagnostic test (Nicolaou 2010). A review of the literature highlights both the paucity of published research that investigates the diagnostic value of Ara h 2-specific IgE concentrations in predicting peanut allergy in peanut-naïve infants and young children; and also the high degree of bias in available studies. Similarly, studies of specific subgroups of allergic children, such as those with eczema or egg allergy, do not exist. Selected studies that fulfilled the QUADAS-2 inclusion criteria are summarised in Table 8 and subsequently discussed individually.

Table 8: Outline of QUADAS-2 scored included studies				
Authors	**Type of study**	**Number of subjects & inclusion criteria**	**Primary outcome**	**Key Findings**
Kim, 2016	Retrospective study of peanut-specific IgE and components in Korean children in two study centres	48 children had OPC (22 proven allergic) 5 children with recent anaphylaxis and detectable specific IgE not challenged	To construct diagnostic decision points for peanut-specific IgE and evaluate the value of Ara h 1,2,3,8&9 in diagnosis	• The 100% PPV for peanut-specific IgE was 10.3kU/L • The 100% PPV for Ara h 2 was 4.0kUA/L • The AUC for Ara h 2 was 0.83 • Measurement of Ara h 2 had no additional benefit
van Veen, 2016	Prospective cohort study of utility of Ara h 1,2,3,6,8 & 9 in 62 children attending a Dutch tertiary clinic between 2012-2013	Children had either a history of previous reaction to peanut or were peanut-sensitised and peanut-naïve.	To evaluate utility of CRD in predicting a) DBPCFC outcomes b) the eliciting dose and c) reaction severity	• Ara h 2 best predictor of peanut allergy but no better at predicting DBPCFC outcome than whole peanut • A negative peanut-specific IgE level to peanut had a 100% NPV removing the need to challenge this subgroup of children • CRD had no value in the prediction of the eliciting dose or the severity of the reaction
Martinet, 2016	Retrospective study of Ara h 2 and Ara h 8 in children attending a French tertiary allergy centre	81/268 children had adequate data recorded including a previous OPC.	To establish a diagnostic decision point for Ara h 2 and Ara h 8 in the diagnosis of peanut allergy	• The optimal cut-off point for whole peanut-SpIgE was 0.5kUA/L; specificity 76% and PPV 85% • For Ara h 2, a cut-off of 1.0kUA/L had a 100% PPV and a 93% NPV • Children with Ara h 2<0.44 kUA/L were at low risk of anaphylaxis and those >14kUA/L were at high risk • Ara h 8 was useful for birch-pollen cross-reactivity
Beyer, 2015	Prospective study of peanut components in German children with suspected peanut allergy	210 children had OPC (90 proven allergic)	To prospectively investigate the role of peanut component-specific IgE in the diagnosis of peanut allergy	• A cut-off value for Ara h 2 of 14.4kUA/L had a 90% probability for peanut allergy • The 95% probability was 42.2kUA/L. • The AUC was 0.92
Ebisawa, 2015	Retrospective study of children attending 2 Japanese centres between 2005-2010	165 children included (35 allergic and 86 tolerant on OPC; 36 with positive history and 8 with negative history	To study the association between SpIgE to peanut and Ara h 2 in the diagnosis of peanut allergy	• Ara h 2 cut-off value of 4.0 kUA/L gave 91.3% PPV • SpIgE concentrations for Ara h 2 were lower than for peanut • There is a relationship between a positive Ara h 2 level and peanut allergy

Table 8: Outline of QUADAS-2 scored included studies (page 2)				
Authors	**Type of study**	**Number of subjects & inclusion criteria**	**Primary outcome**	**Key Findings**
Leo, 2015	Prospective Canadian study of the predictive value of Ara h 2 in Canadian children 2011-2013	137 children included (20/47 failed OPC); 90 were not challenged due to high Ara h 2-SpIgE	To review the clinical utility of Ara h 2-specific IgE concentrations in predicting of peanut allergy in peanut-sensitised children	• 95% PPV for Ara h 2 was between 2 & 3 kUA/L •AUC for Ara h 2 was 0.75 at 0.5 and 0.75 kUA/L •Two patients with negative Ara h 2-SpIgE concentrations failed OPC; one had anaphylaxis •Ara h 2 is an important diagnostic test
Eller and Bindslev-Jensen, 2013	Peanut OPC outcomes correlated with specific IgE concentrations to whole peanut and peanut components Ara h1-3, h8 & h9)	175 peanut OPC positive & 30 OPC negative individuals (aged 1-26; mean age 5.6 years)	To correlate IgE values with OPC outcomes	• Best correlation was found for Ara h2 •Cut-off value for Ara h2 of 1.63 kUA/L yielded specificity of 100% and sensitivity of 70%
Klemans, 2013	Retrospective study of Dutch children with suspected peanut allergy evaluated between 2008 and 2010	100 of 200 eligible patients randomly selected for OPC; 47 allergic, 53 tolerant (DBPCFC n=81; Open n=19)	To develop a new peanut allergy prediction model based on sIgE to components	• Ara h 2 most useful component •Cut-off value for Ara h 2 of 0.35 kUA/L resulted in 88% sensitivity and 84% specificity •The AUC 0.84 •PPV was best at 94%
Lieberman, 2013	Prospective study of 4 cohorts of patients from the US and Sweden	167 children completed an OPC; 106 proved allergic	To evaluate the utility of peanut component testing in the diagnosis of peanut allergy in children with suspected allergy	• Ara h 2 was the best diagnostic test in patients with suspected peanut allergy •May be useful in the reduction of OPCs •PPV 94% •Sensitivity 80% and specificity 92% at a cut-off of <0.35 kUA/L
Lopes de Oliveira, 2013	Prospective study of German children with suspected peanut allergy	Open OPC in 61 children referred to a tertiary centre	To investigate the role of specific IgE concentrations to whole peanut and the components Ara h1,2,3,6&8 in distinguishing peanut allergy from peanut tolerance.	• 94% of allergic and 26% of tolerant children were sensitised to Ara h 2 •Neither whole peanut or Ara h 2 SpIgE concentrations were able to clearly differentiate between allergy and tolerance

Table 8: Outline of QUADAS-2 scored included studies (page 3)

Authors	Type of study	Number of subjects & inclusion criteria	Primary outcome	Key Findings
Surtannon, 2013	Cross-sectional study of peanut sensitised Thai patients between 2008 and 2010	40 peanut-sensitised individuals; clinical reactions determined by clinical history or OPC	To investigate the utility of CRD to differentiate between allergy and tolerance in a low-prevalence country	• Ara h 2 cut-off <0.35kUA/L with sensitivity 68% and specificity 95% • Ara h 2 cut-off of 0.35kUA/L PV 92% and NPV 77% • The AUC was 0.82
Dang, 2012	Prospective Australian birth cohort study of one year old children	Random stratified sample of a population-based birth cohort study of 1-year olds; 100 with peanut allergy and 100 controls (58 of whom were peanut-sensitised). All open challenged	To ascertain whether sIgE to Ara h 2 might improve the accuracy of peanut allergy diagnosis in a general population of infants	• Measuring SpIgE to whole peanut followed by Ara h 2, reduced the number of OPCs by two thirds • Cut-off value for Ara h2 of 1.19 kUA/L detected 60% of children with PA • Cut-off value of 0.10 kUA/L for Ara h2 identified 87% of peanut tolerant children • At a cut-off of 0.10kUA/L, sensitivity was 95% and specificity was 86% • The AUC was 0.95.
Ebisawa, 2012	A prospective study of 57 Japanese children referred for investigation of peanut allergy	57 Japanese children (2-13 yrs) attending specialist allergy clinic with previously primary care diagnosed peanut allergy; 31 proven peanut positive on open OPC & 26 negative	To evaluate utility of IgE to peanut allergens in diagnosis of peanut allergy in Japanese children	• Ara h2 was superior to whole PN • Using the cut-off value of >0.35kUA/L, sensitivity & specificity were 88% and 84% • PPV 82% & unlikely to be useful as a sole tool for diagnosis in the absence of clinical history • AUC 0.91
Nicolaou, 2010	Prospective UK birth cohort study of 8 year old children	A population-based birth cohort of 933 8 year olds tested; 110 (11.8%) sensitised. 19 not challenged (no consent); 12 with known allergy were considered allergic without challenge. 79 children had an OPC and 7 were positive.	•To establish the proportion of sensitised children with true allergy •To investigate the utility of components to distinguish between allergy and tolerance	• Prevalence of true allergy among sensitised 8 year olds estimated as 22.4% • Ara h2 most important predictor • Cut-off value >0.35kUA/L had sensitivity of 1.00 and specificity of 0.96

Legend: ***DBPCFC,*** **Double-blind placebo-controlled food challenge;** ***OPC*****, Oral provocation challenge;** ***PPV*****, Positive predictive value;** ***NPV*****, Negative predictive value;** ***AUC*****, Area under the curve;** ***CRD*****, Component-resolved diagnostics;** ***SpIgE,*** **specific IgE**

Kim, 2016

This retrospective study recruited Korean children with suspected peanut allergy in two centres between 2011 and 2013. The primary aim was to establish diagnostic decision points for whole peanut-specific IgE in the diagnosis of peanut allergy. The secondary aim was to evaluate the clinical utility of measuring specific IgE concentrations to the peanut components Ara h 1,2,3, 8 and 9 in confirming the diagnosis of peanut allergy. The study population was small; 48 children were recruited and 22 reacted upon open oral provocation challenge, the use of the latter inducing a high risk of bias in the patient selection domain according to the QUADAS-2 criteria. An additional 5 children with a clear history of anaphylaxis and detectable whole peanut-specific IgE within the last twelve months were included in the statistical analysis. Bias concerns regarding the index test were low as the study published cut-off values. There was a high level of bias regarding the reference standard domain, which used an unusual protocol - roasting peanuts for ten minutes at 170 degrees. All children, except those excluded for safety reasons, underwent an oral provocation challenge as per routine clinical care and therefore there were no bias concerns regarding study flow and timing. There was a high level of concern regarding study applicability and patient selection, as it is unclear whether all children were tertiary clinic patients.

Summary: Sensitisation to Ara h 2 was higher in peanut allergic children but there were no differences between groups for other components. The 100% positive predictive value was 10.3 kUA/L for whole peanut-specific IgE with a sensitivity of 31.8%, specificity of 100% and negative predictive value of 63.4%. For Ara h 2-specific IgE, the 100% positive predictive value was 4.0 kUA/L. The area under the curve (AUC) for whole peanut-specific IgE antibody levels was 0.91 and for Ara h 2-specific IgE was 0.83. The authors concluded that Ara h 2-specific IgE did not provide any additional diagnostic information and was of little benefit (Kim, 2016).

van Veen, 2016

This prospective cohort study reviewed 62 consecutive children attending a Dutch tertiary allergy clinic between 2012 and 2013. Children had either a history of a previous allergic reaction to peanut, or were peanut-sensitised and peanut-naïve. The study aimed to measure specific IgE concentrations to the peanut components Ara h 1, 2, 3, 6, 8 & 9 and to use these results to predict (a) peanut double-blind placebo-controlled food challenge outcomes, (b) the eliciting dose and (c) the severity of the reaction. The risk of patient selection, index test and reference testing bias was low as subjects were recruited prospectively and were all subjected to a double-blind placebo-controlled food challenge in a single centre within three months of

serological testing. This meant that the risk of flow and timing bias was also low. 33(53%) children reacted with challenge outcomes being strictly scored using Sampson's scoring system. There was a low level of concern with regard to patient selection applicability as children included in this tertiary centre study were either peanut-sensitised and peanut-naïve or had a confirmed history of peanut allergy supported by serological testing. The present thesis examines similar groups of children although refines the study population further to only include children with a history of egg allergy.

Summary: Ara h 2-specific IgE testing was identified as the best predictor of peanut allergy, although peanut component-specific IgE concentrations were no better at predicting double-blind placebo-controlled food challenge outcome, eliciting dose or reaction severity than existing specific IgE or skin prick tests to whole peanut. A negative whole peanut-specific IgE level had a 100% negative predictive value and was superior to that of peanut components. The paper therefore proposed that children with a negative whole specific IgE test need not be subjected to an oral provocation challenge. Components were of no value in predicting either the eliciting dose or the severity of any reaction (van Veen et al., 2016).

Martinet, 2016

This retrospective review of 81 children attending a French tertiary allergy clinic aimed to evaluate the diagnostic decision point for Ara h 2- and Ara h 8-specific IgE testing in the diagnosis of peanut allergy. 81/268 children with adequate data recorded were included in the study analysis. This, combined with the study being retrospective, introduced a high risk of patient selection bias. There was also a high risk of bias for the reference standard as all children were subjected to an open oral provocation challenge, and for flow and timing as it is unclear when children were reviewed. However, bias in the applicability concern domain was low with the majority of patients undergoing an oral provocation challenge scored using the European Academy of Allergy and Clinical Immunology (EAACI) taskforce on anaphylaxis scoring.

Summary: The optimum cut-off value for whole peanut was 0.5kU/L with poor specificity at 76% and a positive predictive value of 85%. Ara h 2-specific IgE level measurements had a 93% negative predictive value and a 100% positive predictive value at a cut-off value of 1.0 kUA/L. An Ara h 2-specific IgE level of <0.44 kUA/L was associated with a low risk of anaphylaxis and a level of >14kU/L with a high risk. Ara h 8 was recommended for evaluation of birch pollen cross-reactivity (Martinet et al., 2016).

Beyer et al, 2015

This study aimed to prospectively investigate the role of peanut and hazelnut components in the diagnosis of German children with suspected allergy being referred to 7 tertiary paediatric hospitals. This review focuses on the peanut allergy section of the study. 210 children with suspected peanut allergy undergoing oral provocation challenges were prospectively recruited. The risk of patient selection bias and index and reference testing bias was low as all children were prospectively challenged, with one third undergoing a double-blind placebo-controlled food challenge rather than an open challenge. There was also a low level of bias within the flow and timing domain as blood was drawn for component testing on the same day as the oral provocation challenge. Specific IgE concentrations were measured for peanut and Ara h 1, 2, 3 and 8. Ninety children (43%) had proven peanut allergy following oral provocation challenge. There was a low level of concern regarding applicability of the study in terms of patient selection, as all patients were tertiary centre patients. It is important that all included study populations are similar to enable comparisons to be made but also, oral provocation challenges are rarely conducted outside the secondary and tertiary setting.

Summary: The best diagnostic test was Ara h 2-specific IgE. A 90% probability for a positive peanut oral provocation challenge was calculated at a cut-off value of 14.4 kUA/L. A 95% probability could only be estimated for Ara h 2-specific IgE concentrations at a cut-off value of 42.2 kUA/L. An area under the curve for Ara h 2-specific IgE was calculated as 0.92 (Beyer, 2015).

Ebisawa, 2015

This study aimed to look at the relationship between whole peanut- and Ara h 2-specific IgE concentrations and peanut allergy in Japanese schoolchildren. A diagnosis of peanut allergy was based on either a positive oral provocation challenge or a convincing positive case history. It is unclear whether the latter was confirmed by testing, placing the study at high risk of patient selection bias as the peanut allergic group is very likely to include children who are not allergic. The risk of bias in the flow and timing domain is unclear as there is no description of the time interval between the index test and the reference standard (oral provocation challenge) taking place although it is likely that the oral provocation challenge was conducted first, given the date of the introduction of component testing. There are concerns regarding patient selection applicability given the study inclusion criteria and high risk of bias together with an unclear level of concern regarding the reference standard, as the scoring criteria are not clearly defined. Therefore the prevalence of peanut allergy in more than 5% of the study population is likely to be misrepresented.

Summary: An Ara h 2-specific IgE cut-off of 4.0 kUA/L had a positive predictive value of 91.3%. For each doubling of specific IgE antibody level, the diagnosis of peanut allergy increased by an odds ratio of 1.74 for Ara h 2- and 1.68 for whole peanut-specific IgE. Ara h 2-specific IgE concentrations were lower than for whole peanut (Ebisawa et al., 2015).

Leo, 2015

This study of 147 peanut-sensitised Canadian children aimed to evaluate whether Ara h 2-specific IgE concentrations are useful in predicting peanut allergy. Recruited children either had a convincing clinical history of peanut allergy or were peanut-naïve. 47 children with an Ara h 2-specific IgE level of ≥10 kUA/L (regardless of whole peanut-specific IgE level) were invited for an open oral provocation challenge; 20 failed (10 with anaphylaxis) and 10 completed the challenge without reaction. This study was at high risk of bias in several domains. In the domain of patient selection bias, patients who were invited for an oral provocation challenge were not selected on the basis of previously published positive predictive values for Ara h 2-specific IgE concentrations as none currently exist; in the reference standard domain, all challenged patients followed an open protocol and in the flow and timing domain, the interval between the index test and reference standard test is unclear. In view of the above, patient applicability concerns also exist as most allergic patients were included on the basis of sensitisation rather than challenge-proven allergy. The scoring system was robust for the classification of challenged children, but this group comprised a small minority of the study population and groups were not analysed separately.

Summary: This study reported that an Ara h 2-specific IgE cut-off value of 0.75 kUA/L (AUC 0.75) was twice as predictive as using a combination of a whole peanut-specific IgE level of more than 2 kUA/L with a positive skin prick test >3mm. Two patients with negative Ara h 2-specific IgE levels failed the oral provocation challenge; one with anaphylaxis (Leo et al., 2015).

Eller, 2013

This paper aimed to correlate specific IgE values to peanut components with peanut oral provocation challenge (OPC) outcomes. Retrospective data from 175 positive and 30 negative OPCs in Danish children and adults aged 1 to 26 years were correlated with specific IgE levels to whole peanut and peanut components.

There was a high risk of selection bias as patients were retrospectively recruited over a six year period (2003-2009). There was also a high risk of bias in the reference standard domain as the majority of OPCs performed were open challenges. Children three years of age or under were

subject to an open challenge (n=165) whilst older children and adults underwent FBPCFC (n=40). Only 158 of the 165 positive OPCs were described as having had objective symptoms. There is no differentiation between results for children and results for adults introducing a high level of concern regarding the applicability of the study into paediatric clinical practice. Another significant limitation of this study is data concerning the small number of subjects proven to be peanut tolerant (30/205).

Summary: This study concluded Ara h 2 to be the most important peanut component in the prediction of peanut allergy with an ODP of 1.63 kUA/L (specificity 1.00; sensitivity >70). The authors reported that using this cut-off value would have reduced the number of OPCs from 205 to 92 concluding that Ara h 2 can significantly improve diagnostic accuracy but cannot completely replace the need for an OPC (Eller and Bindslev-Jensen, 2013).

Klemans, 2013

Klemans compared the utility of Ara h 2-specific IgE testing with an existing model for the prediction of peanut allergy incorporating sex, history, age, skin prick test and specific IgE concentrations to whole peanut and total IgE minus specific IgE to whole peanut. The aim of the study was to improve and update the existing model by adding additional clinical symptoms and peanut component testing. 100 of 200 eligible patients in the Netherlands were randomly selected for inclusion. All patients had been referred with suspected peanut allergy based on a positive history regardless of sensitisation data. This is different to the study population in the present study, which includes only high-risk patients, many of whom are peanut-naive. There is a high level of concern regarding patient selection applicability, as the patient study group in this paper is not directly comparable with the subgroup population of high-risk atopic infants examined in the present study. It may however, be comparable with the tertiary clinic population as a whole in terms of the evaluation of children referred with suspected peanut allergy. All children were challenged; 81 underwent double-blind placebo-controlled food challenges and 19 (mostly younger) children were subject to open challenges.

Summary: The discriminative ability of Ara h 2-specific IgE in predicting peanut allergy was found to be comparable with the updated prediction model. This adds to the accruing evidence suggesting that Ara h 2-specific IgE is the most useful peanut component in the diagnosis of peanut allergy (Klemans, 2013).

Lieberman et al, 2013

Lieberman et al compared whole peanut-specific IgE concentrations with the recombinant allergens Ara h 1, 2, 3 and 8 in 167 children with suspected peanut allergy undergoing an oral peanut provocation challenge in the US or Sweden. There was a high level of patient selection bias with individuals being recruited from four different cohorts within the two countries, with varying degrees of suspicion of peanut allergy. This also introduces a high level of concern regarding study applicability under the patient selection domain.

All patients were subjected to an oral provocation challenge, which in the majority were double-blind placebo-controlled food challenges, and blood was taken immediately prior to the procedure. Overall, 63.5% patients reacted. Specific IgE concentrations to components and whole peanut were compared between peanut allergic and tolerant groups. Subgroup analysis between the four cohorts was also conducted. Ara h 2-specific IgE was the most specific test for peanut allergy with specificities ranging from 85% to 95% between subgroups.

Summary: Ara h 2-specific IgE was the most specific test among the combined groups with the best positive predictive value (0.94). Receiver-operating characteristic curve analysis demonstrated an area under the curve of 0.84. Ara h 2-specific IgE may have a role in the reduction of the number of oral provocation challenges required in clinical practice and is particularly of value when combined with whole peanut-specific IgE testing to peanut in a stepwise approach (Lieberman, 2015)

Lopes des Oliveira et al, 2013

Lopes des Oliveira et al investigated the diagnostic value of specific IgE to whole peanut and the components Ara h 1, 2, 3, 6 and 8 among 61 German children. The risks of patient selection bias and level of applicability concern were both low. All children had been referred to a tertiary centre and all were challenged regardless of whole peanut-specific IgE level. The index test of Ara h 2-specific IgE concentration measurements was different in this study to all of the others as it was ascertained by using the ISAC chip microarray immunoassay. This is a solid-phase assay that enables the measurement of specific IgE antibodies to multiple allergenic components using only 20µ of serum. There was a high risk of bias with the reference standard as all oral provocation challenges were open challenges. 25 children had a whole peanut-specific IgE level to above the published cut-off value of 15kUA/L, although 7(28%) of these were peanut tolerant on oral provocation challenge giving a false positive rate of 28%. 34/61(56%) children were proven allergic. 94% of peanut allergic children were sensitised to Ara h 2-specific IgE although 26% of tolerant patients were also sensitised.

Summary: Ara h 2-specific IgE measurements did not clearly differentiate between clinical allergy and clinical tolerance and reliance upon the test is likely to lead to misclassification. An oral provocation challenge remains necessary in order to make an accurate diagnosis (Lopes de Oliveira, 2013).

Suratannon et al, 2013

This study aimed to investigate the role of component-resolved diagnostics in the evaluation of peanut allergy among peanut-sensitised individuals in Thailand. There was a high risk of patient selection bias as individuals were included based on peanut sensitisation and clinical history alone. The sample size of only 40 patients (19 proven allergic and 21 proven tolerant) was small. Double-blind placebo-controlled food challenges were only performed if oral provocation challenge symptoms were subjective, which introduced a high risk of bias in the reference standard domain. It is unclear how many of the study children were challenged and how many had a diagnosis based on history and sensitisation alone, which identifies a high risk of flow and timing bias.

Summary: Ara h 2-specific IgE was the most prevalent component in individuals with peanut allergy (68%) but was lower than in many other studies. Ara h 2-specific IgE was also associated with peanut allergy and anaphylaxis. A ratio of Ara h 2- to whole peanut-specific IgE was associated with the identification of children at risk of anaphylaxis. At a cut-off of <0.35kUA/L, sensitivity was 68% and specificity was 95%. The area under the curve was 0.82 (Surtannon, 2013).

Dang et al, 2012

This study investigated the role of measuring Ara h 2-specific IgE concentrations in the reduction of oral food challenges for the diagnosis of peanut allergy. Children received skin prick testing to peanut, followed by whole peanut- and Ara h 2-specific IgE testing. Skin prick testing was regarded as positive if the measured wheal diameter was ≥1mm. This is an unusually low cut-off value. The study utilised a population-based cohort of one-year old Australian infants and included 100 infants with peanut allergy and 100 peanut tolerant infants, 42 of whom were non-peanut sensitised atopic controls leading to a high risk of patient selection bias. There is little indication for measuring Ara h 2-specific IgE concentrations in non-peanut sensitised atopic controls as these infants do not require any further clinical evaluation given that the negative predictive value of routine whole peanut testing is so good. Such children are unlikely to be undergoing clinical review for potential

peanut allergy in a tertiary allergy clinic. As a population-based birth cohort, these children would not all be at risk of allergic disease, and so these results cannot be extrapolated into the clinical environment. QUADAS-2 identifies this as a high level of concern regarding the applicability of the findings to clinical practice. All children were one year of age, which tells us little about the sensitisation profile in children from other age groups. Additionally, population-based cohorts do not reflect tertiary clinical practice, as the pre-test probability is different to those children routinely encountered in clinical practice. There was a high risk of bias in the reference standard domain as the scoring system for mild reactions was weak and could have led to the inclusion of non-allergic infants.

Summary: A cut-off value of 1.19kUA/L for Ara h 2-specific IgE testing was more accurate in predicting peanut allergy than skin prick testing or specific IgE testing to whole peanut, and could potentially reduce the number of oral provocation challenges by two thirds. A negative predictive cut-off value of 0.10 kUA/L identified 87% of peanut-tolerant infants (Dang et al., 2012).

Ebisawa et al, 2012

This study reported a consecutively recruited cohort of 57 Japanese children, aged two to thirteen years, with previous doctor-diagnosed peanut allergy undergoing a peanut oral provocation challenge. All children had either a positive clinical history or positive sensitisation and were recruited consecutively. The initial peanut allergy diagnosis had been given in primary care and was largely based on self-reported symptoms. Some of the children included in the peanut-tolerant group were likely to have never been previously allergic and therefore not eligible for inclusion in this study, creating a high level of concern in the patient selection domain. 26(46%) children were proven peanut-allergic, and 54% proven to be peanut-tolerant on oral provocation. There was a high risk of bias in the reference standard domain as all oral provocation challenges were open challenges. Using the manufacturer's cut-off value of <0.35 kUA/L, Ara h 2-specific IgE was demonstrated to be the superior diagnostic test with a sensitivity of 88% and a specificity of 84%. The area under the curve was 0.91. Specificity was increased to 94% when Ara h 2-specific IgE testing was performed in conjunction with testing to Ara h 1 and h 3.

Summary: Japanese children are frequently sensitised to the peanut component Ara h 2, which performed better than whole peanut-specific IgE concentrations for the diagnosis of peanut allergy. The optimal decision point was 0.66 kUA/L (Ebisawa, 2012).

Nicolaou et al, 2010

This study measured whole peanut-specific IgE concentrations in a population-based birth cohort of 933 8 year-old children to ascertain whether peanut allergy could be distinguished from peanut sensitisation. As with the HealthNuts study, there were significant limitations to using the findings of a population-based birth cohort in clinical practice, which raises a high concern regarding the applicability of the reference standard to secondary or tertiary care. Studies that include children without a clinical suspicion of peanut allergy are at high risk of patient selection bias by virtue of the fact that they focus on individuals who would not routinely be investigated for peanut allergy. 110(11.8%) children were sensitised to peanut using the conventional test. 12 with a convincing clinical history of a previous reaction were considered peanut allergic without further investigation. 79 children underwent oral provocation challenge; 7 of whom were proven peanut allergic. More than 50% of oral provocation challenges were open rather than being a double-blind placebo-controlled food challenge. This potentially introduces a high risk of reference standard bias due to the potential for observer bias. Peanut allergic subjects had increased sensitivity to peanut components Ara h1, 2 and 3. Peanut tolerant subjects were primarily sensitised to pollen allergens.

Summary: The prevalence of peanut allergy among peanut-sensitised children was estimated to be 22.4%. The majority of children considered peanut-sensitised using standard tests do not have true peanut allergy. A positive Ara h 2-specific IgE concentration was the most important predictor of true allergy (Nicolaou et al., 2010).

2.4 Minor studies excluded by QUADAS-2

Excluded papers with poor methodological quality were identified. Six select articles that did not fulfil the designated eligibility criteria following QUADAS-2 scoring but which are notable are summarised in Table 9 and described briefly below.

Table 9: Outline of useful minor studies which were excluded by QUADAS-2 scoring review

Authors	Reason for exclusion	Type of study	Number of subjects & inclusion criteria	Primary outcome	Key Findings
Grabenhenrich, 2016	This study population was previously presented in the paper by Beyer et al described in Table 8 and discussed above	Prospective 9-multicentre study, using data previously published	207 children undergoing their first clinical review for exclusion or confirmation of peanut or hazelnut allergy	To examine whether the ratio of component or specific IgE measurements to total serum IgE concentrations could improve the prediction of OPC outcome	• Ara h 2 best diagnostic test with AUC of 0.93 • Calculation of ratio measures was unhelpful
Agabriel, 2015	Only 15% of study population were subject to an OPC which is below the QUADAS-2 cut-off for inclusion	Peanut component population profile study	Children not subjected to an OP had a diagnosis based on history and SPT or SpIgE (but PPV not used so high risk of false positive results)	To investigate peanut component profile of French Mediterranean children with suspected PA	• Ara h 6 best predictor of PA • Ara h 2 cut-off value 0.13kUA/L (PPV 86%) • AUC 0.78 for Ara h 6 and 0.74 for Ara h 2
Ballmer-Weber, 2015	The atopic controls included were pollen-sensitised individuals not a comparable cohort	Peanut component population profile study in 11 European countries	68 PA individuals and 82 atopic (pollen-sensitised) and non-atopic controls 28 of 54 challenges were DBPCFC Combination of children and adults	To study molecular peanut sensitisation patterns among individuals with PA	• Ara h 2 major allergen in children presenting under 14 yrs • Ara h 2 SpIgE level of ≥1.0kUA/L had 97% probability of a systemic reaction
Trendelenburg, 2014	No control group No cut-off values published Weak criteria for categorisation of OPC outcome	Retrospective study in a German tertiary allergy clinic 2007-2011	53 peanut-naïve sensitised children with eczema identified but clinical relevance data only available on 24 (45%)	To investigate an allergen profile of peanut-sensitised peanut-naïve infants and young children with eczema and assess clinical relevance	• Ara h 1 was the immunodominant allergen, followed by Ara h 2 and 3 respectively • Ara h 2 was not detected in 43% of children with proven peanut allergy
Keet, 2013	Unclear whether patients were randomly or consecutively included	Retrospective review in a single US centre	60 children: 26 with a history of peanut allergy and 35 peanut-sensitised, peanut-naïve children.	To assess the applicability of previously published Ara h 2-specific cut-off values to a general paediatric allergy clinic population.	• There were high misclassification rates with most cut-off values. • Ara h 2 did not replace an allergy-focussed history with oral provocation challenge
Codreanu, 2011	Case-control study utilising non-comparable controls	A case-control study in two French allergy clinics	166 patients with proven PA 61 pollen sensitised peanut tolerant patients 10 non-allergic controls	To investigate the diagnostic performance of SpIgE to peanut components to reduce need for OPC	• Ara h 2 had 96% sensitivity & 85% specificity with a cut-off value of 0.10 • Optimal decision point of 0.23 gave 93% sensitivity & 96% specificity • Measurement of Ara h 2 increased test specificity and reduced OPC referrals

***Legend. OPC,* oral provocation challenge; *PPV*, Positive predictive value; *NPV*, Negative predictive value; *AUC*, Area under the curve; *CRD,* Component-resolved diagnostics**

Grabenhenrich, 2016

This was a prospective multicentre study of 207 children undergoing their first evaluation for confirmation or exclusion of peanut or hazelnut allergy in nine German centres. The aim was to examine whether the ratio of either component or specific IgE measurements to total serum IgE concentrations could improve the prediction of oral provocation challenge outcome in children. Both peanut and hazelnut-specific IgE concentrations were measured together with the relevant components although the hazelnut data is excluded from this present review. The study population was the same study population previously included in the paper by Beyer et al below (Beyer, 2015).

Summary: The best diagnostic test was Ara h 2-specific IgE testing with an area under the curve (AUC) of 0.93. The probability of a positive peanut provocation challenge was 16%. The calculation of ratio measures failed to improve diagnostic prediction. Cut-off values were those reported separately by Beyer et al (Beyer, 2015, Grabenhenrich et al., 2016).

Agabriel et al, 2015

This study of 181 children referred to a French tertiary allergy clinic with suspected peanut allergy aimed to describe the component profile of their Mediterranean population. There was a high risk of bias in their patient selection with only 15% of the study population being subjected to an open food challenge. Other children were categorised as allergic on the basis of a recent positive clinical history of a reaction and either a positive skin prick test of 4mm or a specific IgE level to whole peanut above 1 kUA/L, which is very low and increases the risk of false positives.

Summary: French Mediterranean children had a lower prevalence of seed storage protein sensitisation than other studied populations. The highest prevalence of 64% was to Ara h 6, followed by Ara h 2 at 63%. The best predictor of peanut allergy was Ara h 6- (positive predictive value 96%) followed by Ara h 2-specific IgE testing (positive predictive value 86%) at a cut-off value of 0.11kUA/L. The area under the curve was 0.78 for Ara h 6 and 0.74 for Ara h 2-specific IgE concentrations. The predictive performance of peanut was not discussed. Ara h 2-specific IgE testing was not found to be discriminant with respect to the development of tolerance (Agabriel, 2014).

Ballmer-Weber et al, 2015

This study included 68 peanut allergic and 82 atopic and non-atopic controls from 11 European countries in a study of molecular peanut sensitisation patterns among peanut allergic individuals – the EuroPrevall study. The study additionally examined age at onset of allergy. Allergy was confirmed by double-blind placebo-controlled food challenge in 28 of 54 challenged subjects with no history of previous anaphylaxis. There was a high risk of selection bias as subjects were acquired via mixed cohorts from varied background settings. Additionally, the atopic controls had never been suspected of having peanut allergy but were pollen-sensitised individuals. Among those 54 individuals who did undergo a double-blind placebo-controlled food challenge, Ara h 2-specific IgE testing had a sensitivity of 45% and a specificity of 100%.

Summary: Ara h 2 was the major allergen recognised by the peanut allergic subjects. Sensitisation to Ara h 1 and 2 almost exclusively occurred in children who presented with allergy prior to 14 years of age. Ara h 8 or 9 were the predominantly recognised allergens among tolerant subjects and adult peanut allergic individuals. Subjects with Ara h 2-specific IgE concentrations of ≥1.0 kUA/L had a 97% probability of experiencing a systemic reaction (Ballmer-Weber, 2015).

Trendelenburg, 2014

This study aimed to investigate the component-resolved diagnostic profile of peanut sensitisation in peanut-naïve infants and young children with eczema attending a German tertiary allergy centre, and to assess its clinical relevance. This was a retrospective study and ineligible for inclusion as it had no control group. It also had a high risk of bias in both domains, as it produced no cut-off values and did not clearly score oral provocation challenge outcome. The study is notable and relevant to this thesis as it is the only study which evaluated a particular subgroup of peanut-naïve peanut-sensitised children; those with eczema. The present thesis evaluates a different subgroup, children with egg allergy.

Summary: Seed storage proteins were the immunodominant allergens in this study population, with Ara h 1 being recognised in the majority of children followed by Ara h 2 and then Ara h 3. However, Ara h 2 was not detected in 43% of children with proven peanut allergy (Trendelenburg et al., 2014).

Keet, 2013

This US study was a retrospective review of 60 children who had been subjected to an open diagnostic peanut oral provocation challenge between 2003 and 2010. It aimed to investigate the applicability of published Ara h 2-specific IgE cut-off values to a general paediatric allergy clinic population. There were two groups of children included; 26 with a positive clinical history and 34 who were peanut-sensitised and peanut-naïve. 26 children reacted on oral provocation challenge but it is not clear to which group they had been assigned prior to oral provocation challenge. One child was challenged twice, and passed a second oral provocation challenge two years after failing the first. The risk of patient selection bias was high as it is not specified how children were selected. It is unclear whether only children with banked serum were included or whether inclusion was appropriately random or consecutive. The use of banked serum may lead to patient selection bias as serum may only be available for select groups of patients rather than random inclusion. This has the potential consequence that the reported diagnostic outcome measures may not accurately reflect the study population as a whole as if all eligible subjects had been included. There was also bias in the use of the index test as the study did not identify and report its own cut-off values. There was a high risk of bias with the index reference standard as it was performed several years after the oral provocation challenge and used banked samples. In addition, children were challenged with a non-standardised peanut-containing food of their preference.

Summary: The study reported a high rate of misclassification when applying previously published Ara h 2-specific IgE concentrations to their general paediatric allergy clinic population: 26% at 0.23 kUA/L, 21% at the manufacturer's cut-off 0.35 kUA/L, 36% at 2.0 kUA/L and 21% at 0.3 kUA/L. Children also reacted on oral provocation challenge with a negative Ara h 2-specific IgE level below 0.35 kUA/L. Ara h 2-specific IgE testing was not able to replace an allergy-focussed clinical history in conjunction with an oral provocation challenge (Keet et al., 2013).

Codreanu et al, 2011

Codreanu et al evaluated the diagnostic performance of specific IgE to peanut components. Specific IgE to whole peanut and peanut components were measured in 3 groups of patients; those with proven peanut allergy (n=166), pollen-sensitised peanut tolerant patients (n=61) and non-allergic controls (n=10). This study was not included as it was a case-control design using non-comparable controls, who have never been investigated for suspected peanut allergy and therefore at high risk of patient selection bias. This includes controls who regularly consume peanut without reaction. The best predictor of peanut allergy was found for Ara h 2-

specific IgE testing which was 96% sensitive and 85% specific, with a cut-off value of 0.10 kUA/L. An optimal decision point of 0.23 kUA/L gave 93% sensitivity and 96% specificity. Unfortunately results were combined for children and adults, making the results less easily extrapolated to a purely paediatric population.

Summary: The measurement of Ara h 2-specific IgE can be used to diagnose peanut allergy with an acceptable degree of precision, increasing test specificity and reducing oral provocation challenges for the majority of children (Codreanu et al., 2011).

2.5 Synopsis of Review of Relevant Articles

Component-resolved diagnostics (CRD) is a new and evolving field. Published optimal decision points for Ara h 2-specific IgE concentrations do exist but vary considerably between studies. Nicolaou et al suggest the prevalence of peanut allergy among peanut sensitised children within a general population birth cohort study to be low at 22.4%, which intensifies the need to find an accurate screening tool to predict clinical peanut allergy with acceptable sensitivity and specificity (Nicolaou et al., 2010a). All studies were prone to bias, primarily within the patient selection domain as inclusion criteria varied between studies. Some studies included children who were peanut-sensitised without any clinical history of a reaction and others included children with both a clinical history and peanut sensitisation. Other studies included patients who reported peanut allergy but failed to confirm it via serological or skin prick testing. Many studies included children with peanut skin prick or specific IgE test values above a commonly published positive predicted value primarily for safety reasons, although it should also be noted that parents are frequently unwilling to consent to an oral provocation challenge with significantly elevated test results. Additionally, many studies were conducted retrospectively. There was often also bias within the reference standard domain as many studies operated open oral provocation challenges rather than double-blind placebo-controlled food challenges. However, this is likely to be because open challenges are routine in clinical practice with double-blind placebo-controlled food challenges being more common in a research setting. The measurement of Ara h 2-specific IgE concentrations was proposed as the best test for the diagnosis of peanut allergy in almost all selected studies although there was some variation between sensitivity (80 to 100%) and specificity (60-97%) as shown in Table 8.

A systematic review of the diagnostic accuracy of specific IgE components in diagnosing peanut allergy was conducted by Klemans et al (2013) and based on the diagnosis of allergy in those patients suspected of being peanut allergic. The aim was to establish how to best manage those individuals with either a positive clinical history regardless of sensitisation or

individuals who were peanut-sensitised but peanut-naïve. This population varies from that within the present study, which aims to quantify the management of a specific high-risk group of peanut-sensitised atopic children – those with egg allergy. The pre-test probability of being peanut allergic within this cohort of infants will be higher than that addressed by Klemans. However, Klemans reported that studies with different inclusion criteria were demonstrated to have very little variation in their sensitivity and specificity values (Klemans, 2015).

The selected studies described above are not directly comparable. Despite this, in the majority of studies study, Ara h 2-specific IgE testing was found to have the best diagnostic accuracy compared with the standard diagnostic tests of whole peanut-specific IgE testing or skin prick testing. However, the more recent studies by van Veen et al and Kim et al in 2016 did not find Ara h 2-specific IgE testing to be superior to whole peanut-specific IgE testing in predicting oral provocation challenge outcome (van Veen et al., 2016, Kim, 2016). Many studies also investigated other peanut components, which proved to be of limited clinical value. Martinet et al described Ara h 8 to be useful for identifying birch pollen cross-reactivity (Martinet et al., 2016). Several studies published diagnostic cut-off specific IgE and SPT values, although the criteria for establishing these cut-off points varied between studies. Some studies used 90% or 95% positive predictive values which are dependent on the composition of the study population, whereas others used the specific IgE level representing the 95% specificity of the test which is not dependent upon prevalence of the disease. Cut-off values are depicted in Table 10 and are presented with associated published data such as optimal decision points where available. Some studies looked at more than one cut-off value and therefore appear in the table more than once.

The majority of included studies were conducted in secondary or tertiary allergy centres. 5 of these were within Europe, 2 in the United States, 4 in Asia, 1 in the UK, 1 in Australia and 1 in Canada. Despite this geographical variation, Ara h 2-specific IgE testing remained the optimal clinical test. Apart from one study, all used the same method to measure whole peanut-specific IgE concentrations (ImmunoCap, Thermofisher, Sweden). Ara h 8-specific IgE testing was of some value in two studies but remained suboptimal compared with Ara h 2-specific IgE testing and was best suited to identifying birch pollen cross-reactivity. Most of the European studies were from northern Europe and it would be interesting to establish if Ara h 2 is the predominant allergen in southern Europe, given that several papers have identified the LTP protein Ara h 9 as an immunodominant allergen in this population. However, patients under review in the current study in the West of England are UK infants and very few are of southern European descent.

Table 10: Summary table of the clinical utility of specific IgE for peanut component allergens from included studies

First author, Year	Cut-off (kUA/L)	Area under the curve (AUC)	Sensitivity (%)	Specificity (%)
Beyer, 2015	0.1	0.92	86	86
Lieberman, 2015	0.35	0.84	80	92
Eller, 2013	0.35	-	89	60
Eller, 2013	1.28	0.90	91	72
Klemans, 2013	0.35	0.90	91	72
Lopes de Oliveira, 2013	0.30	-	94	74
Suratannon, 2013	0.35	0.82	68	95
Dang, 2012	0.1	0.95	95	86
Dang, 2012	0.35	-	81	88
Ebisawa, 2012	0.35	0.91	88	84
Nicolaou, 2010	0.35	0.99	100	96
Nicolaou, 2010	0.23	-	93	97
Nicolaou, 2010	1.28	0.90	76	97

Legend. *Optimal cut-off point. Some studies reported more than one cut-off point.

2.6 Conclusions following review of relevant articles

There are important clinical implications to finding an improved measure for predicting a positive reaction on oral peanut provocation challenge as described above. Many young children undergo oral provocation challenges in order to ascertain whether they have definitive peanut allergy rather than mere peanut-sensitisation. The current diagnostic approach comprises the taking of a detailed allergy-focussed clinical history combined with the measurement of either skin prick tests or whole peanut-specific IgE measurements, with current diagnostic techniques being unacceptably inadequate in children (Eigenmann and Sampson, 1998).

In routine clinical practice at Bristol Royal Hospital for Children in 2014, 280 oral provocation challenges were performed. As described above, 53(19%) of these were to peanut, following routine whole peanut-specific IgE and peanut skin prick testing in the paediatric outpatient clinic. Despite following evidence-based, published diagnostic guidelines, only 4(8%) of these peanut oral provocation challenges were positive. The low positive rate suggests that many children may not be being fully investigated and that there appears to be a high degree of clinical reticence to challenge children with a positive skin test. It is likely that there are many children, labelled as peanut allergic, who actually are tolerant. A peanut oral provocation challenge is currently the only definitive way to ascertain a child's current peanut allergy status. The role of Ara h 2-specific IgE testing may be an important one; it may allow clinicians to change their clinical practice to ensure that those children who are extremely likely to react are not challenged, whilst also encouraging clinicians to fully investigate those children within the immunological grey area rather than simply labelling them as peanut allergic and running the risk of misclassification. If proven to be extremely valuable in the diagnosis of peanut allergy in high-risk children, then the measurement of Ara h 2-specific IgE concentrations may be able to replace the oral provocation challenge for a significant proportion of children.

Protecting children from harm is of paramount importance. Despite clear, published guidelines on performing a food peanut oral provocation challenge, these do imply a potential risk to children and incur the potential risk of provoking a life-threatening allergic reaction (Sampson, 2001). There are also important issues for service delivery and staffing concentrations. Waiting lists for peanut oral provocation challenges can be many weeks long and establishing a reliable and simple clinical test to reduce the number of children waiting for tests would be valuable, particularly from a health-economics perspective. It is important that such a screening test has sufficient sensitivity and specificity to ensure that peanut tolerant children do not miss the opportunity of being offered a peanut oral provocation challenge that would demonstrate that

they do not have peanut allergy, and that allergic children are not placed at unnecessary risk. Clearly the development of positive predictive values will be helpful in the management of egg and peanut allergic children within the allergy clinic setting. A positive predictive value of 95% suggests that there is no need to subject a child for a peanut oral provocation challenge as they are highly likely to react with less than 5% of children who will be falsely labelled as allergic. Ethical approval to challenge this group of children would also be difficult to secure. Positive predictive values did vary between the studies discussed above although some studies concurred. Sampson's positive predictive values for the measurement of whole peanut-specific IgE are perhaps the most frequently quoted, and were confirmed by Roberts and Lack in 2005, who found them to be generalizable to different populations of children undergoing clinical review (Roberts and Lack, 2005). It is notable however, that not all studies found this 95% cut-off value useful and in one study, the misclassification rate for the diagnosis of peanut allergy was high with 25(28%) of peanut tolerant children having a whole peanut-specific IgE level of greater than 15kUA/L (Lopes de Oliveira, 2013). This study did not look at higher positive predictive values such as those proposed by other studies. Dang's study calculated the misclassification rate utilising these same positive predictive values and found it to be extremely low at only 3% or less (Dang et al., 2012). 43(43%) peanut allergic children had a skin prick test wheal diameter of <8mm which placed them in what is commonly regarded as 'the immunological grey area', necessitating a provocation challenge. As described above, up to 43% of children with a skin prick test of less than 8mm will still react on oral provocation challenge, but this is currently the only way to give a child a definitive diagnosis. The use of an improved diagnostic test would be very useful in clinical practice within Bristol Children's Hospital. Standards for the management of egg allergic children, who are at high-risk of peanut allergy, are currently not clearly outlined, with clinicians of varying levels of seniority managing children in different ways according to their own preferred criteria.

This study will investigate the hypothesis that specific sensitisation to the peanut component Ara h 2 is associated with a diagnosis of clinical peanut allergy in egg-allergic children. Consequently, Ara h 2-specific IgE testing may have a better diagnostic performance than whole peanut-specific IgE testing in predicting which children will react during their oral provocation challenge. This may ultimately mean that unnecessary oral provocation challenges in children with peanut allergy could be potentially reduced or avoided.

Chapter 3

Methods

Tom, the Hot Tamales Man, and his horse drawn refreshment wagon, USC, 1910

Reynolds, John, comp. The Trojan Gallery: A Pictorial History of the University of Southern California, p. 46. Los Angeles: University of Southern California, 1980. LD5101.S32T76 (USC Libraries). http://digitallibrary.usc.edu/cdm/ref/collection/p15799coll104/id/604

CHAPTER 3

METHODS

3.1 Aims and objectives

1. To investigate the diagnostic value of Ara h 2-specific IgE measured in serum in predicting a clinical reaction to peanut in peanut-sensitised, peanut-naïve children with a history of egg allergy.

2. To evaluate whether the measurement of Ara h 2-specific IgE concentrations is clinically useful in the management of peanut-naïve, egg allergic children who are sensitised to peanut.

3. To develop a post hoc diagnostic algorithm model for the use of Ara h 2-specific IgE.

3.2 Objectives

- Recruit a population of egg allergic children with sensitisation to peanut;
- Perform whole peanut- and Ara h 2-specific IgE testing, and skin prick testing to peanut prior to peanut oral provocation challenge;
- Define two groups of children with clinical peanut allergy versus clinical tolerance on the basis of either oral provocation challenge outcome or skin prick test wheal diameter above previously published 95% positive predictive values for peanut allergy;
- Include an additional control group of egg allergic children with previously confirmed peanut allergy;
- Compare the performance of whole peanut- and Ara h 2-specific IgE testing and peanut skin prick testing in the diagnosis of clinical peanut allergy in egg allergic children;
- Develop a diagnostic algorithm to optimise the clinical utility of all available tests.

3.3 Null hypothesis

The hypothesis of the study is that there is no relationship between a positive Ara h 2-specific IgE antibody concentration and clinical peanut allergy in a cohort of high-risk egg allergic children.

3.4 Study Design

This was a prospective, observational study comparing the measurement of Ara h 2-specific IgE concentration testing in addition to routine conventional whole peanut-specific IgE concentration testing. Children with a history of egg allergy who were sensitised to peanut were subject to an oral peanut provocation challenge as per usual care. The oral provocation challenge outcomes were related to peanut- and Ara h 2-specific IgE concentrations and to skin prick test wheal diameters.

3.5 Recruitment strategy

Parents or carers of eligible children were informed about the study by an Allergy Clinical Nurse Specialist as outlined below:

1. Where possible, parents of all eligible children were sent a Parent Information Sheet through the post in advance of their clinic appointment (Appendix 1: Parent Information Sheet; Appendix 2: Child Participant Information Sheet)
2. The Clinical Nurse Specialist discussed the study with the family on arrival at the clinic.
3. If the child had egg allergy and peanut sensitisation on skin prick testing and the parent was happy for them to participate in the study, written informed consent was taken prior to routine blood testing.

A copy of the consent form was filed in the patient records and the original was given to the parents (Appendix 3: Consent Form). A letter to the GP was sent informing them of the child's participation in the study (Appendix 4: GP Information Letter).

3.6 Subjects

All peanut-sensitised, egg allergic children aged between 12 months and 17 years of age referred to the Paediatric Allergy Clinics at Bristol Royal Hospital for Children for review of egg allergy over an eighteen month period were invited to participate in the study. Those willing to participate and whose parent or guardian gave informed consent were enrolled in the study. The period of recruitment ran from January 2015 to June 2016. Inclusion was performed consecutively.

All children had either ongoing egg allergy or a previous confirmed diagnosis of egg allergy that had resolved, and a positive peanut skin prick test or whole peanut-specific IgE concentration. Egg allergy was confirmed by a positive clinical history of a convincing type I hypersensitivity reaction to egg and a recorded positive specific IgE level or skin prick test to egg white.

Peanut-naïve children with a whole peanut-specific IgE concentration or skin prick test wheal diameter of less than previously published 95% positive predictive values for peanut allergy of ≥15kUA/L and ≥8mm were each referred for a peanut oral provocation challenge in line with routine clinical practice to determine their current peanut allergic status (Sampson and Ho, 1997, Sporik et al., 2000). Children with positive predictive values above the 95% cut-off point were allocated into the peanut allergic group. Children with known peanut allergy confirmed by positive whole peanut-specific IgE testing also had Ara h 2-specific IgE concentrations measured but did not undergo an oral provocation challenge but were included and analysed separately.

3.7 Inclusion and exclusion criteria

Children were eligible if they had a history of egg allergy confirmed by either a positive skin prick test to egg or a positive egg white-specific IgE concentration above 0.35kUA/L, and sensitisation to whole peanut-specific IgE or skin prick testing together with written, informed parental consent. Children were excluded if they had any chronic disease requiring intervention or therapy with the exception of the atopic co-morbidities, wheeze, asthma, eczema, other food allergy or rhinitis. Children without any confirmed documentation of a previous egg allergy were also excluded.

3.7.1 Comparator groups

Children with known and confirmed peanut allergy

In addition to peanut-naïve infants and children, children undergoing review who had a history of egg allergy with confirmed and documented peanut allergy were also included. These children had peanut allergy confirmed by skin prick test or whole peanut-specific IgE testing supported by either a positive food challenge in hospital or a documented clinical allergic reaction to peanut. Data from these children were included in some of the analyses but this subgroup were also analysed separately. When analysed separately these children were allocated to the known peanut allergy subgroup.

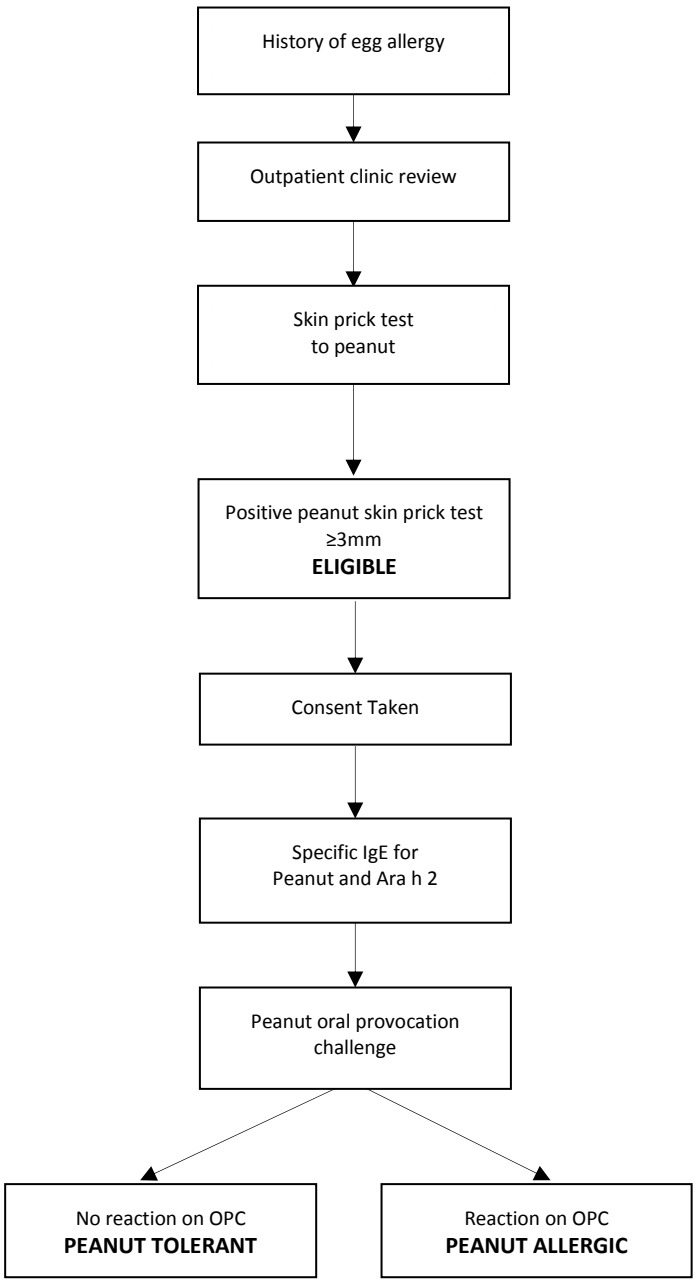
Children identified with resolved peanut allergy

There was a small subgroup children who had been referred to clinic for review of possible peanut allergy resolution and who passed their oral provocation challenge without reaction, despite continuing to have positive whole peanut-specific IgE concentrations or skin prick tests. All these children had been reviewed in this centre previously where peanut allergy had been confirmed and documented. One of these children had previously failed a peanut oral provocation challenge two years previously with clear documentation of this in their medical notes. These children were allocated to the peanut allergy resolved group.

3.8 Study visits

The study required two patient visits as shown in Figure 10 below.

Figure 10: Flowchart to show patient visits and investigations



Children attending the outpatient clinic were subject to skin prick testing to peanut and venepuncture to facilitate the measurement of total IgE concentrations and concentrations to whole peanut- and Ara h 2-specific IgE. Written informed consent was taken from parents and carers at this initial visit, and assent forms were also completed where applicable. Children

who had a whole peanut-specific IgE concentration of less than 15kU/L were invited for a peanut oral provocation challenge as per routine care. Outcome measures are listed in Table 11.

Table 11: Outcome measures

Method of measurement:	Description:
Sensitisation to whole peanut via skin prick test	Wheal diameter of ≥3mm to whole peanut
Sensitisation to peanut via whole-peanut specific IgE antibody concentration	≥0.35 kUA/L – Positive according to manufacturer's instructions
Sensitisation to Ara h 2 via Ara h 2-specific IgE antibody concentration	≥0.35 kUA/L – Positive according to manufacturer's instructions
Peanut allergy status	As confirmed by oral provocation challenge or clear recent clinical history

3.9 Measurement of whole peanut-specific and Ara h 2-specific IgE in serum

Total IgE, whole peanut-specific IgE and Ara h 2-specific IgE were determined by singleplex ImmunoCAP fluorescence enzyme immunoassay (Thermofisher Scientific, Uppsala, Sweden). Serum samples from all children were taken at the outpatient review by a paediatric phlebotomist, using the standard paediatric technique of using a tourniquet on either the child's upper or lower arm and drawing blood using a retractable butterfly from the antecubital fossa or the back of the hand. Samples were collected into a gold-top serum-separator tube. An additional 0.5ml serum was taken for the measurement of Ara h 2-specific IgE antibody concentrations in addition to routine whole peanut-specific IgE testing. Samples were left to clot prior to being centrifuged for 15 minutes. Samples were subsequently aspirated into aliquots and stored at -20˚C. During processing, the allergen placed within the assay reacts with any specific IgE in the individual serum sample. Non-specific IgE is then washed away and enzyme-antibodies against IgE are added to create a complex. Following incubation, any unbound enzyme-anti-IgE is washed away and the bound complex is incubated further with a developing agent. This is then completed by the introduction of a stopping solution and the fluorescence of the remaining product is measured and transformed to a standard concentration using a calibration curve (Phadia, 2014). Specific IgE was measured in kilounits of antibody per litre (kUA/L) with a lower detection limit of 0.35 kUA/L and a maximum limit of 100 kUA/L and results were recorded. Values $\geq$ 0.35 kUA/L to whole peanut and to Ara h 2 were considered positive according to the manufacturer's instructions and recorded as a continuous variable.

3.10 Skin Prick Testing

Allergen skin prick tests were performed using a commercial one-prick lancet technique, using commercially available extracts of peanut using a 1:20 (wt/vol) solution (Soluprick, ALK, Uppsala, Sweden). All tests were performed by a specialist paediatric allergy nurse. Histamine dihydrochloride (10mg/mL, ALK-Abello A/S, Horsholm, Denmark) was used as a positive control and saline (Soluprick SQ, ALK-Abello) was used as a negative control. Skin tests were performed on the volar aspect of the forearm. The maximal skin wheal diameter (mm) was measured with a ruler after 15 minutes and recorded. A reaction was considered positive if the resultant wheal was ≥3mm in diameter in the presence of a reaction to histamine of at least 3mm in diameter and a negative response to the saline negative control. A skin prick test wheal diameter of ≥3mm has been recommended as a marker of hypersensitivity to foods in many North American and European centres.(Dreborg, 1993, Bock et al., 1977).

3.11 Sensitisation to peanut

All children had either skin prick testing and/or specific IgE antibody concentrations measured to peanut. Both tests were performed in almost all children except in select cases were children were unable to discontinue their antihistamines or where the laboratory had an insufficient sample for full analysis. Ara h 2-specific IgE and total IgE concentrations were also measured. Children were considered peanut sensitised and eligible for study inclusion if they had a skin prick test wheal diameter of 3mm or greater and/or a specific level to peanut of ≥0.35 kUA/L according to the manufacturer's instructions. Specific IgE concentrations were measured between 0.35 and 100 kUA/L. For the purposes of statistical analysis concentrations below <0.35 KUA/L were recorded as 0.34 and those >100 were recorded as 101 kUA/L.

3.12 Oral provocation challenge

Children were referred for a routine open peanut oral provocation challenge in the usual manner. The decision to challenge was made as per routine clinical practice by the physician or specialist nurse in the allergy clinic and was not affected by the child's participation in the study. Challenges are indicated when an allergy-focussed clinical history together with skin prick or whole peanut-specific IgE testing gives insufficient information for the reviewing clinician to diagnose an individual with peanut allergy or peanut tolerance. Challenges occurred between one and five months later due to the length of the food challenge waiting list and were performed under medical supervision on the day care unit at Bristol Royal Hospital for Children. Open challenges, where the family are aware that each challenge dose contains peanut, were performed in preference to double-blind placebo-controlled food

challenges due to both clinical service pressures and because this study was an evaluation of routine clinical care outcomes.

All eligible children were challenged with either ground peanuts or Bamba peanut snack (peanut puff crisps). Both foods contain demonstrable Ara h 2 (James Hindley, Indoor Biotechnologies Limited, personal communication, June 11 2010, European Academy of Allergy and Clinical Immunology, Vienna). Ground peanut was mixed in 110g Petit Filous chocolate dessert (Yoplait, Uxbridge). For all subjects the initial dose was 0.25g of peanut. The highest dose was 10g for children aged 3 years or younger and 15g for older children. For Bamba, doses ranged between 0.85g and 17g for children 3 years or younger and 34g for older children. Doses were given every 15 minutes. Patients were observed on the paediatric day care ward for two hours after the final dose. Reactions occurring within one hour were considered immediate and reactions occurring after one hour were considered late reactions. Challenge doses are shown below in Table 13 and protocols are included in Appendix 5. One peanut contains approximately 200mg of protein (Goldman, 1998).

Results were recorded as positive (any two objective allergic symptoms on or within 2 hours) or negative (no reaction). Symptoms were recorded and classified by body system and in terms of severity using a validated scoring system (Bock et al., 1988). Reactions were classified into skin, upper and lower respiratory, gastrointestinal and cardiovascular reactions. Children with a positive reaction to peanut following oral provocation challenge were allocated to the Peanut Allergic group whilst those proven peanut tolerant were allocated to the Peanut Tolerant group. The outcomes of the challenges were related to the serum concentration of specific IgE antibodies to whole peanut and Ara h 2.

Table 12: Oral peanut provocation challenge doses

Ground peanut	Bamba peanut snack
0.25g	0.85g
0.5g	2.13g
2g	4.25g
4g	8.50g
10g	17.00g
15g	34.00g

Legend. Bamba is a soft peanut puff which can be easily fed to very young children, and can be mixed with water to create a weaning paste.

3.13 Classification of Symptoms

There are many symptom classification systems for type I hypersensitivity reactions. The first study to classify symptoms was devised by Mueller for the grading of systemic allergic reactions to insect venom (Mueller, 1959)**.** This system has been commonly used either in its original form or adapted for use in descriptions of food anaphylaxis. In this study, reactions were scored using the Oral Food Challenge Symptom Score Sheet in Appendix 6 (Bock et al., 1988). This scoring chart is straightforward for use by allergy ward staff and ensures that each symptom can be easily recorded. It also ensures that oral challenges are not discontinued due to subjective symptoms.

3.14 Data recording

Skin prick test results, whole peanut- and Ara h 2-specific IgE concentrations and peanut provocation challenge outcomes were recorded on an anonymised, password-protected database, kept in a locked office. Hard copies of all paperwork were kept in a separate location.

3.15 Ethics and R&D Approvals

The University of Bath undertook the study sponsorship role. The Proportionate Review Sub-Committee of the Camden and Islington Research Ethics Committee granted ethical Approval for the study in October 2014. The University of Bath Research Ethics Approval Committee for Health and the UHBristol Research & Innovation Department approved the study protocol (Appendix 7). This study was conducted in accordance with the Research Governance Framework for Health and Social Care and Good Clinical Practice.

3.16 Provision for dealing with attrition

It was anticipated that some families would choose not to have a peanut provocation challenge and that others might fail to complete the entire peanut provocation challenge. Recruited children who elected not to proceed with the challenge were removed from the final analysis as were children whom the managing clinician elected not to challenge. Other anticipated reasons for attrition were lost or insufficient samples for full analysis. It was decided to include children if they had an Ara h 2-specific IgE measurement available together with one other test result for whole peanut; either a skin prick test or a whole peanut-specific IgE measurement.

3.17 Power Calculation

Previous studies have published various optimal decision point cut-off values for predicting peanut allergy using both whole peanut and various peanut components. Calculations using the primary outcome measure of Ara h2-specific IgE concentrations are based on a study by Dang et al 2012, which reported a mean (SD) Ara h2-specific IgE concentration of 7.11 kUA/L (11.49) in peanut allergic Australian infants and a mean (SD) Ara h2-specific IgE concentration of 0.25 kUA/L (0.53) in peanut tolerant infants (Dang et al, 2012). The minimal important difference was therefore calculated as 6.86. Using these figures and an employing a 1-sided significance level, for the study to have 80% power to detect the minimal important difference a total of 72 patients would be required. Assuming an attrition rate of 10%, 80 patients would need to be recruited. A secondary analysis looked at receiver operating characteristic (ROC) curves, constructed to test the difference between the predictive ability of skin prick test wheal diameters to peanut, and whole peanut and Ara h 2-specific IgE concentrations.

Null Hypothesis: **There is no relationship between a positive Ara h 2-specific IgE antibody concentration and clinical peanut allergy in a cohort of high-risk egg allergic children.**

3.18 Analysis

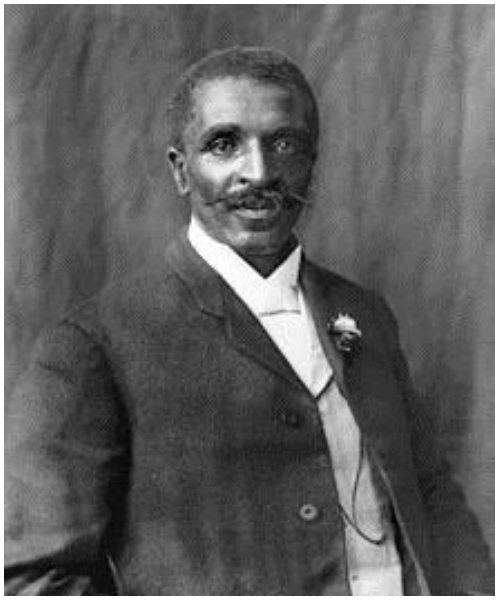
Analyses were conducted to compare the likelihood of a positive peanut oral provocation challenge following each of the three screening tests: Skin prick test to peanut, whole peanut-specific IgE concentration and Ara h 2-specific IgE concentration testing. Skin prick test wheal diameters were normally distributed and differences between groups were examined using analysis of variance (ANOVA). Specific IgE concentrations were not normally distributed continuous variables and the Kruskal-Wallis test was used for non-parametric group comparisons. Differences in median specific IgE concentrations were analysed using the Mann-Whitney *U* test and dichotomous variables (such as peanut allergy status) using Fisher's Exact test. A p value of <0.05 was considered statistically significant for all tests.

Logistic regression analysis was performed to ascertain the effects of Ara h 2-specific IgE concentrations on the likelihood of a child having peanut allergy. Receiver-operator characteristic curves were constructed. An area under the curve of greater than 80% suggests that the test is a good diagnostic test with clinical utility. In practice this means that if a clinician were to take two children, one allergic and one tolerant and perform the selected test

on them, then the child with the abnormal test result should be the child from the peanut allergic group. 2 by 2 contingency tables were used to calculate sensitivity and specificity for specific IgE and skin prick test cut-off values. Likelihood ratios were calculated to assist the clinician to calculate an individual child's post-test probability of having peanut allergy. Statistical analyses were conducted with IBM SPSS Statistics for Macintosh, Version 22 (Armonk, NY). Data are presented as mean ± standard deviation (SD).

Chapter 4

Results Section 1: Data Presentation



George Washington Carver, 1906.

Carver promoted alternative food crops to enable poor farmers to grow alternative crops as a source of their own food and of other products to improve their quality of life. He wrote 105 recipes that included peanuts. He also developed and promoted more than 100 products made from peanuts useful for the house and farm, including cosmetics, dyes and plastics.
This image is available from the United States Library of Congress' Prints and Photographs division under the digital ID ppmsc.03252.

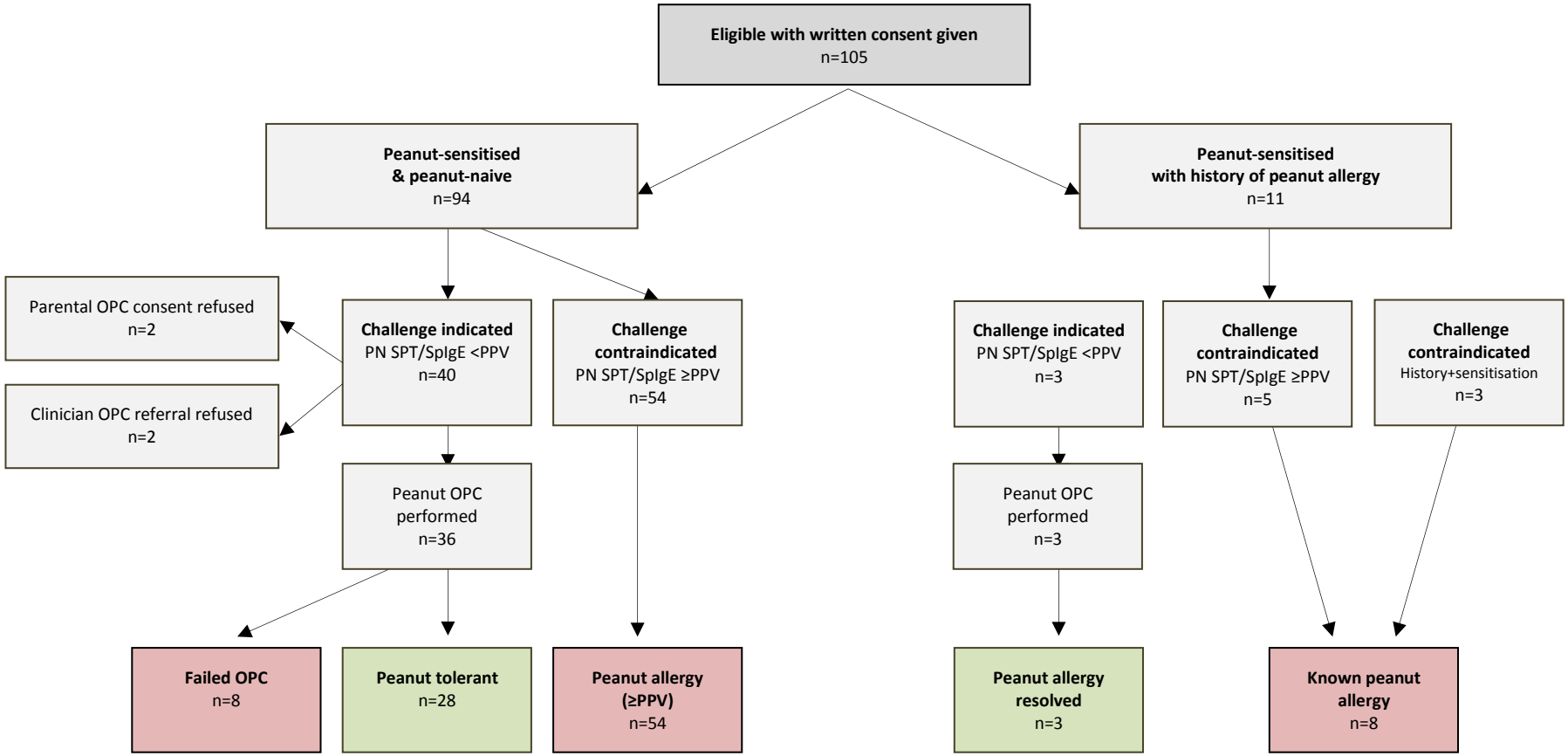
CHAPTER 4

RESULTS SECTION 1: DATA PRESENTATION

4.1 Subjects

During the study period 105 peanut-sensitised, peanut-naïve children (with consent provided by a parent or guardian) were enrolled and listed for a peanut oral provocation challenge as per routine care (Figure 11). The parents of an additional child, whose peanut allergy status was known and for whom all data was available, declined to give their consent for inclusion of their results and outcome in the study. This may have been due to a language barrier. 54 children with a whole peanut-specific IgE test value, or skin prick test wheal diameter equivalent to, or above previously published positive predictive values of 15kUA/L or 8mm were not challenged. Additionally, 11 egg allergic children had a previous documented allergic reaction to peanut confirmed. Four of 105 children were not included in the final analysis; two children did not undergo an oral provocation challenge due to parental anxiety and two children with results below the positive predictive value were not challenged as per their managing consultant's clinical decision. In a smaller centre such as Bristol, there is some variability between clinicians regarding their clinical management decisions. 101 children were therefore included in the study analysis. 36 children were subject to a peanut oral provocation challenge. No children had inconclusive challenges. There were two sets of siblings included. 58 children were male and 43 were female. 14 infants were less than 2 years of age and 87 children were aged 2 years of age and above. The pathway of the subjects through the study is shown in Figure 11.

Figure 11: Pathway of subjects through the study protocol



***Legend. Peanut allergic children are shown in pink and peanut tolerant children are shown in green. OPC*, oral provocation challenge; *PPV*, positive predictive value; *SPT*, skin prick test; *SpIgE*, Specific IgE, *PN*, Peanut.**

4.2 Outcome groups

The children were divided into two outcome groups – peanut allergic and peanut tolerant. Each group was subdivided into further outcome groups as described in Table 13; challenge-proven peanut allergy, values above previously published 95% positive predictive values, known peanut allergy and resolved peanut allergy. Throughout the results chapter, the focus will be upon the analysis of the two primary groups of allergic and tolerant groups of children. Analysis will also be made of combinations of subgroups.

Table 13: Outcome groups

Outcome group	Children (n)	Mean age (yr)[range]	Gender M:F
Total Peanut Tolerant:	**31**	**6.6 [1.1-17.9]**	**16:15**
Resolved Peanut Allergy	*3*	*8.2 [5.7-11.4]*	*1:2*
Total Peanut Allergic:	**70**	**6.4 [1.2-16.0]**	**42:18**
De Novo Peanut Allergic:	62	6.2 [1.2-13.5]	38:14
Challenge proven allergy	*8*	*5.7 [1.2-13.0]*	*3:5*
Values ≥PPV	*54*	*6.2 [1.9-13.7]*	*35:19*
Known Peanut Allergy	8	8.2 [1.4-12.0]	4:4

Legend. Primary outcome groups are shown in bold.

4.2.1 Children with known peanut allergy

11 peanut-sensitised egg-allergic children reported a history of a previous reaction at home. Previous confirmation by either a peanut skin prick test or whole peanut-specific IgE concentration was available. Only one child had contacted acute emergency services. 10(90%) were treated with antihistamine. No children had been treated with Salbutamol or intramuscular adrenaline. One child was particularly unwell with urticaria, reduced consciousness, and recurrent diarrhoea and vomiting for several hours. This child was not brought for medical help and did not take any rescue medications. The precipitating dose is more difficult to quantity for this group of children although 8(73%) children reported reactions following contact with a small amount of peanut. All reactions occurred within 1 hour of ingestion and no child experienced a delayed reaction. One child did experience a prolonged reaction but this was largely due to lack of medical intervention.

4.2.2 Children with resolved peanut allergy

Three children with a previous history of peanut allergy confirmed by previous clinical correspondence and previous positive whole peanut-specific IgE testing completed an oral provocation challenge without reaction. One of these children had failed a peanut oral provocation challenge two years previously in our centre. These children were all subject to a routine peanut oral provocation challenge due to falling levels of whole peanut-specific IgE with no reaction over the last two years.

4.3 Reactions on peanut oral provocation challenge

36 children completed a peanut oral provocation challenge. 28 children completed the challenge without reaction whilst 8 demonstrated Type I hypersensitivity symptoms. 8 children had symptoms on oral provocation challenge, with the provoking dose ranging between 0.25g and 4g. Reactions were recorded using a validated symptom score system which classifies reactions by body system into skin, upper and lower respiratory, gastrointestinal and cardiovascular reactions. Reactions were assessed and managed by a ward allergy support nurse under the supervision of an Allergy Specialist Nurse or a Consultant Paediatrician specialising in allergy. All symptoms developed within 20 minutes of the previous ingested dose and resolved within the two-hour post challenge observation period. There were no late phase reactions and no child experienced a delayed reaction following discharge. All children received oral antihistamines; one child was prescribed additional Prednisolone by an inexperienced junior doctor due to lack of response to antihistamine. No children required Salbutamol or intramuscular adrenaline. Reactions on oral provocation challenge are summarised in Table 14.

Table 14: Scored reactions on oral provocation challenge

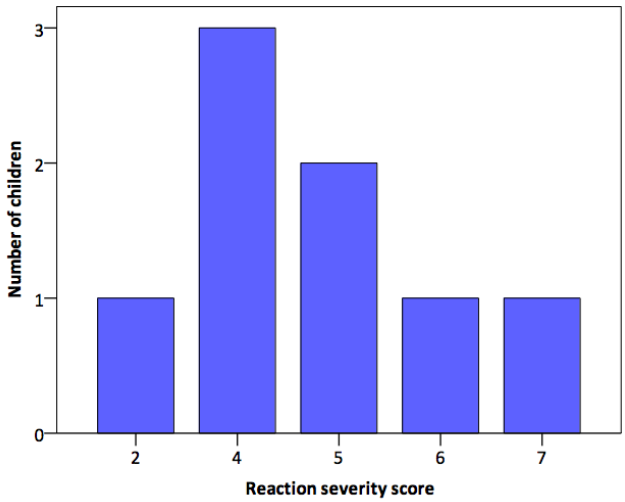
Subject	Age (years)	Type	Stage	Symptoms	Score	Treatment
1 (M)	13	Peanut	1	Facial urticaria; Oral pruritus	2	Cetirizine
2 (F)	2	Peanut	2	Perioral erythema; Vomit x2; Contact urticaria on hands	4	Cetirizine
3 (F)	1.3	Bamba	1	Moderate rhinorrhoea; Hives x4; Facial erythema; Conjunctival pruritus; Cough;Fractious	6	Cetirizine
4 (F)	6	Peanut	2	Vomit x 2; Moderate abdominal pain	4	Cetirizine
5 (M)	6	Bamba	4	Facial urticaria x1; Sneezing; Conjunctival pruritus **1 hr Post Cetirizine:** Nausea; Diarrhoea x5; Widespread urticarial rash	7	Cetirizine Prednisolone
6 (M)	3.5	Peanut	1	>3 Perioral hives; Oral pruritus; Excessive drooling	4	Cetirizine
7 (M)	4.5	Bamba	1	Widespread urticaria; Pruritus	5	Cetirizine
8 (F)	7.5	Bamba	3	3 Hives; Vomit x 1; Rhinorrhoea; Minimal cough	5	Cetirizine

Legend. Reactions were scored using a validated scoring system published by Bock et al (Bock et al., 1988)

Figure 12 depicts the severity of reactions. The most common symptoms elicited occurring in 88% of children were cutaneous. 4 (50%) children developed gastrointestinal symptoms. Most children reacted early in the peanut oral provocation challenge although one child reacted after the fourth dose and then continued to develop further symptoms despite being given oral antihistamine. The mean dose provoking a reaction was 1 gram. This does not differ from threshold doses published in the literature (Taylor et al., 2009).

No child experienced clear cardiovascular, respiratory or laryngeal symptoms although two children were allocated positive scores under the laryngeal criteria for occasional cough. Of these, one young child who complained of oral symptoms had extreme excessive drooling but was too young to be able to describe his symptoms clearly. His only other symptom was mild facial urticaria. Another infant developed significant rhinorrhoea and conjunctival symptoms together with minimal facial urticaria (4 hives) and erythema. This child was reported to have minimal cough although no wheeze. It was hard to ascertain whether the child was coughing due to oral symptoms or whether the cough resulted from airway compromise as no other airway signs were present and her oxygen saturations were normal. This child was too young to be able to define her symptoms clearly.

Figure 12: Severity score of allergic symptoms in children undergoing a peanut oral provocation challenge



Legend: Severity was classified according to a validated scoring system which classified reactions in terms of severity, increasing in severity from 1 to 7.

4.4 Primary analysis of peanut allergic and peanut tolerant children

The primary analysis within this study is to make comparisons between the two groups of peanut allergic and peanut tolerant children for the three tests: peanut skin prick testing, and measurement of whole peanut- and Ara h 2-specific IgE concentrations. Table 16 shows median whole peanut- and Ara h 2-specific IgE concentrations and mean skin prick test wheal diameters to peanut for each outcome group. Each test will subsequently be described separately.

4.4.1 Skin prick test wheal diameters compared with peanut oral provocation challenge outcomes for peanut allergic and peanut tolerant children

The primary outcome of this study is to examine the clinical utility of Ara h 2-specific IgE between groups of peanut allergic and peanut tolerant children. As skin prick testing is the most frequently utilised test in the paediatric allergy clinic, it is necessary to examine its clinical utility as a basis for comparison with Ara h 2-specific IgE. Skin prick test reactivity to peanut was therefore examined in relation to peanut oral provocation challenge outcomes in 97 children. The differences in wheal diameters for peanut allergic and peanut tolerant children are demonstrated in Figure 13.

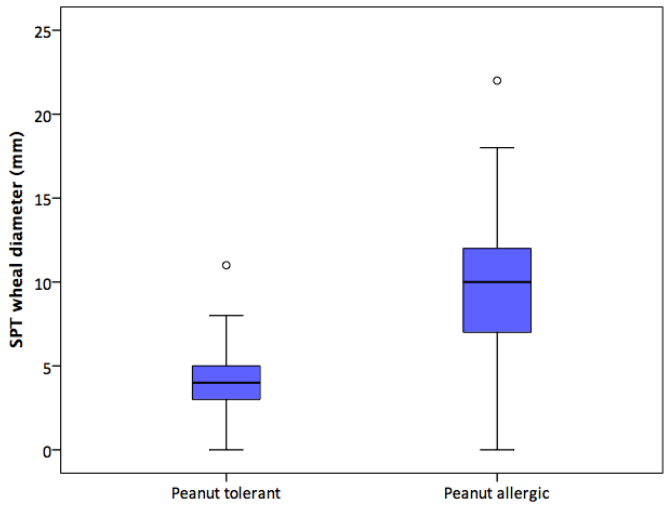
Table 15. Skin prick test results, whole peanut, Ara h 2 and total IgE concentration grouped by oral provocation challenge outcome

Outcome group	Children (n)	Mean age (years)[range]	Mean skin prick test (mm) [range]	Median Peanut- Specific IgE [range]	Median Ara h 2-Specific IgE [range]	Median Total IgE [range]
Total Peanut Tolerant:	**31**	**6.6 [1.1-17.9]**	**4.0 [0-11]**	**1.0 [0.34-51.6]**	**0.34 [0.34-1.5]**	**302 [6-4584]**
Resolved Peanut Allergy	*3*	*8.2 [5.7-11.4]*	*4.3 [3-5]*	*0.38 [0.34-0.47]*	*0.34 [0.34-101]*	*219.5 [142-297]*
Total De Novo Allergy + Known Allergy	**70**	**6.4 [1.2-16.0]**	**9.6 [0-22]**	**11.6 [0.34-101]**	**4.8 [0.34-101]**	**359 [11-11702]**
De Novo Peanut Allergic:	62	6.2 [1.2-13.5]	4.1 [0-22]	11.6 [0.34-101]	4.8 [0.34-101]	359 [11-11702]
Challenge proven allergy	*8*	*5.7 [1.2-13.0]*	*4.4 [0-11]*	*1.2 [0.34-2.55]*	*0.34 [0.34-2.7]*	*114.5 [32-3949]*
Values ≥PPV	*54*	*6.2 [1.9-13.7]*	*10.62 [0-22]*	*18.0 [0.34-101]*	*8.5 [0.34-101]*	*1032 [19-4129]*
Known Peanut Allergy	8	8.2 [1.4-12.0]	7.0 [4-13]	47.8 [0.34-101]	28.2 [0.53-101]	380 [11-11702]

Legend: Children were divided into subgroups of allergic and tolerant children.

Peanut tolerant children had a mean wheal diameter to peanut of 4mm (SD± 2.5, range 0-11) whilst peanut allergic children had a mean wheal diameter of 10mm (SD± 4.1, range 0-22). There is no overlap of the interquartile ranges, reflecting a real difference between the two groups. Whilst the number of children with a positive skin prick test result to peanut above the manufacturer's cut-off value of 3mm was not significantly different between groups (67/67 (100%) *vs* 25/30 (83%) (Fisher's Exact p=45.11), 2 (7%) tolerant children had a skin prick test wheal diameter above the positive predictive value compared with 47 (70%) of allergic children. This was confirmed to be significant (Fisher's Exact test p<0.05).

Figure 13: Peanut skin prick test diameters in peanut allergic and peanut tolerant children



Legend: The study population was divided into two primary outcome groups; those who were peanut allergic and those who were peanut tolerant. Skin prick test wheal diameter was significantly larger in peanut allergic children (Fisher's Exact test; p<0.05)

4.4.2 Whole-peanut and Ara h 2-specific IgE concentrations in two groups of peanut allergic and peanut tolerant children

In order to establish whether Ara h 2-specific IgE is a useful test clinically, a comparison was made between whole-peanut specific IgE and Ara h 2-specific IgE concentrations (Figure 14).

Whole-peanut specific IgE concentrations

Whole peanut-specific IgE concentrations were available for 99 subjects and were collected as per routine care. 83(84%) subjects had both positive skin prick test results and elevated whole peanut-specific IgE concentrations ≥0.35kUA/L. 22(22%) children had discordant results: 13(13%) subjects were sensitised on skin prick testing but not on whole-specific IgE testing; 9 of these children proved peanut tolerant. 3(3%) subjects were sensitised on specific IgE level testing only; 2 were peanut tolerant.

Ara h 2-specific IgE concentrations

Ara h 2-specific IgE concentrations were available for 101 children. 5 of 8(63%) children with challenge-proven peanut allergy had negative Ara h 2-specific IgE concentrations. 58(57%) children had positive Ara h 2-specific IgE concentrations of ≥0.35 kUA/L; of these only 2(3%) were peanut tolerant. 1 child with challenge-proven peanut allergy had discordant results having a positive Ara h 2-specific IgE concentration and a negative whole peanut-specific IgE concentration.

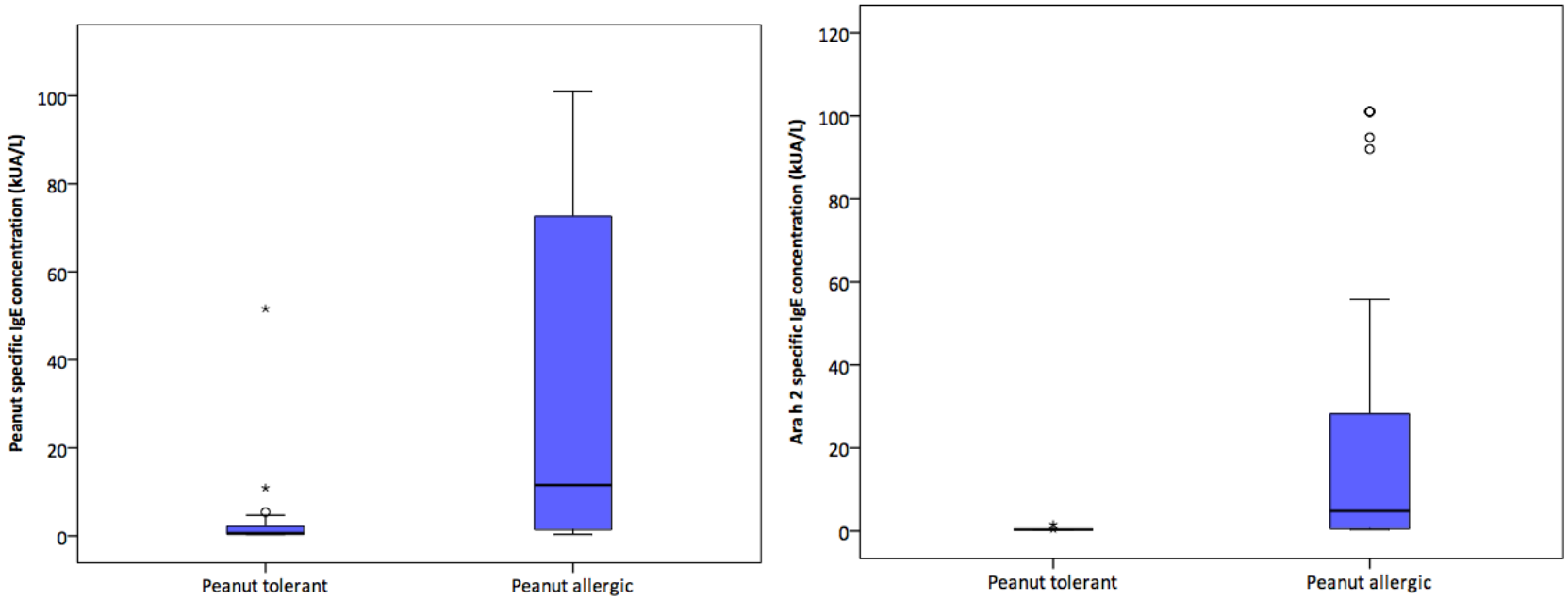
Comparison of whole peanut- and Ara h 2-specific IgE concentrations

Whole peanut- and Ara h 2-specific IgE concentrations are presented in Figure 14. The range of detectable whole peanut-specific IgE was greater in peanut allergic than peanut tolerant children with the median being 11.6 kUA/L (IQR 74.4, 0.34-101). For peanut tolerant children the median was less at 0.63 kUA/L (IQR 1.98, 0.34-51.6). Ara h 2-specific IgE concentrations were lower than those for whole peanut. The median Ara h 2-specific IgE for tolerant children was 0.34 kUA/L (IQR 0, 0.34-1.54) compared with 4.8 kUA/L (IQR 28.4, 0.34-101) for allergic children.

Figure 14: Comparison between whole peanut-specific IgE and Ara h 2-specific IgE concentrations in peanut allergic and tolerant children

A) whole peanut-specific IgE

B) Ara h 2-specific IgE



Legend: The study population was divided into two primary outcome groups; those who were peanut allergic and those who were peanut tolerant. Whole peanut and Ara h 2 specific IgE concentrations were significantly higher in peanut allergic children (Fisher's Exact test; p<0.05

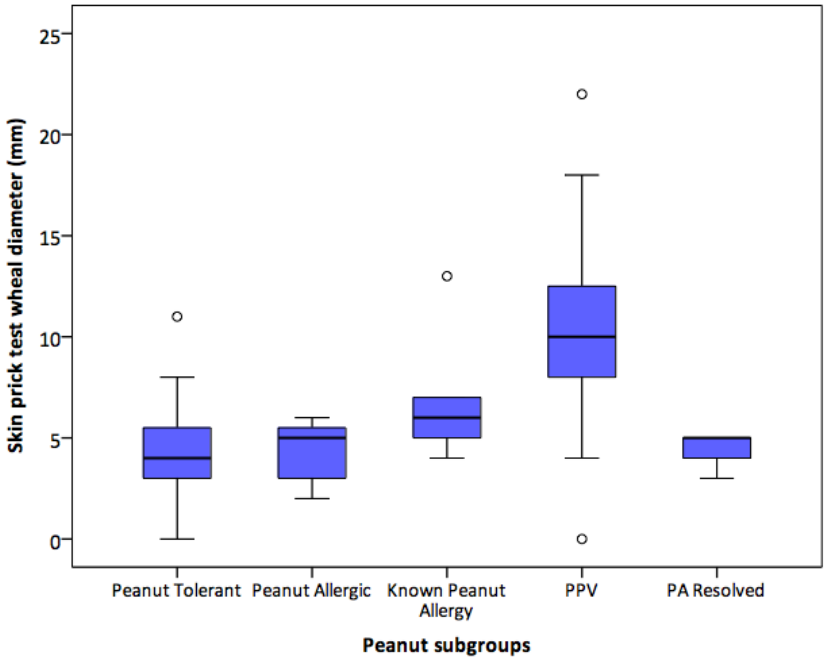
4.5 Secondary analysis of subgroups of peanut allergic and peanut tolerant children

The secondary analysis within this study is to make comparisons between the subgroups of peanut allergic children; those with challenge-proven peanut allergy, those with whole peanut-specific IgE or skin prick test values above previously published positive predictive values and those who have experienced a previous confirmed allergic reaction to peanut. The most clinically useful finding would be to identify tests able to distinguish between children with challenge-proven allergy or tolerance.

4.5.1 Subgroup analysis of the clinical utility of peanut skin prick test wheal diameters in the prediction of peanut allergy status

Figure 15 depicts differences in skin prick test wheal diameters between the separate oral provocation challenge outcome subgroups. The mean peanut wheal diameter was 4mm (SD±1.5, range 2-6) for children in with challenge-proven peanut allergy, 7mm (SD±3.5, range 4-13) for children with known peanut allergy, 11mm (SD±3.7, range 0-22) for children with values above the positive predictive value, 4mm (SD±2.6, range 0-11) for tolerant children and 4mm (SD±1.2, range 3-5) for children with resolved allergy. A one-way ANOVA demonstrated that at least one group was significantly different to the others [$F(4,90)=22.36$, $p<0.0001$]. Post hoc multiple pairwise comparisons using the Bonferroni correction indicated that the mean wheal diameter for children within the positive predictive value subgroup was significantly different than the mean wheal diameter for children in the challenge-proven peanut allergy or challenge-proven peanut tolerance subgroups. There was no significant difference for any other group comparisons (Figure 15).

Figure 15: Peanut skin prick test wheal diameters according to oral provocation challenge outcome group



Legend: Peanut allergic and tolerant children were further divided into subgroups; Challenge-proven peanut tolerance, challenge-proven peanut allergy, known peanut allergy, positive predictive value or resolved peanut allergy. The mean wheal diameter for children within the positive predictive value subgroup was significantly higher than children with challenge proven allergy or tolerance.

4.5.2 Subgroup analysis of whole peanut- and Ara h 2-specific IgE concentrations compared with peanut oral provocation challenge outcomes

Whole-peanut specific IgE concentrations

A Kruskal-Wallis test was performed to examine differences between the subgroups depicted in Figure 16; challenge-proven peanut allergic, known peanut allergy, test values above previously published 95% positive predictive values, challenge-proven peanut tolerance and resolved peanut allergy. This demonstrated that at least one group was significantly different from the others ($p<0.0001$). Median whole peanut-specific IgE values were highest in children with known peanut allergy at 47.8 KUA/L (IQR 99.1, 0.70-101) and in children in the positive predictive value subgroup at 18 KUA/L (IQR 38.9,0.34-101). However, there was no clear difference in median whole peanut-specific IgE values between children who were tolerant and children with challenge proven peanut allergy. The median whole peanut-specific IgE was 1.0 KUA/L (IQR 2.7,0.34-51.6) for tolerant children and 1.2 KUA/L (IQR 1.44,0.34-2.55) for challenge proven allergic children. Outliers existed in the tolerant group rather than the allergic group.

A series of post hoc Mann-Whitney U non-parametric tests were performed to compare differences between subgroups. Median whole peanut-specific IgE were significantly higher for children in the positive predictive value subgroup than for children with challenge-proven peanut allergy (U=55.5, z=-3.321, p<0.0001), resolved peanut allergy (U=7.0, z=-2.636, p<0.01) or peanut tolerance (U=222.5, z=-5.110, p<0.0001). There was also a significant difference in median whole peanut-specific IgE between children with known peanut allergy and challenge-proven peanut allergy (U=9.0, z=-2.423, p<0.05) or tolerance (U=35.0, z=-2.954, p<0.005). Applying the Bonferroni correction identifies that only values less than 0.005 were significant as ten pairwise comparisons were made.

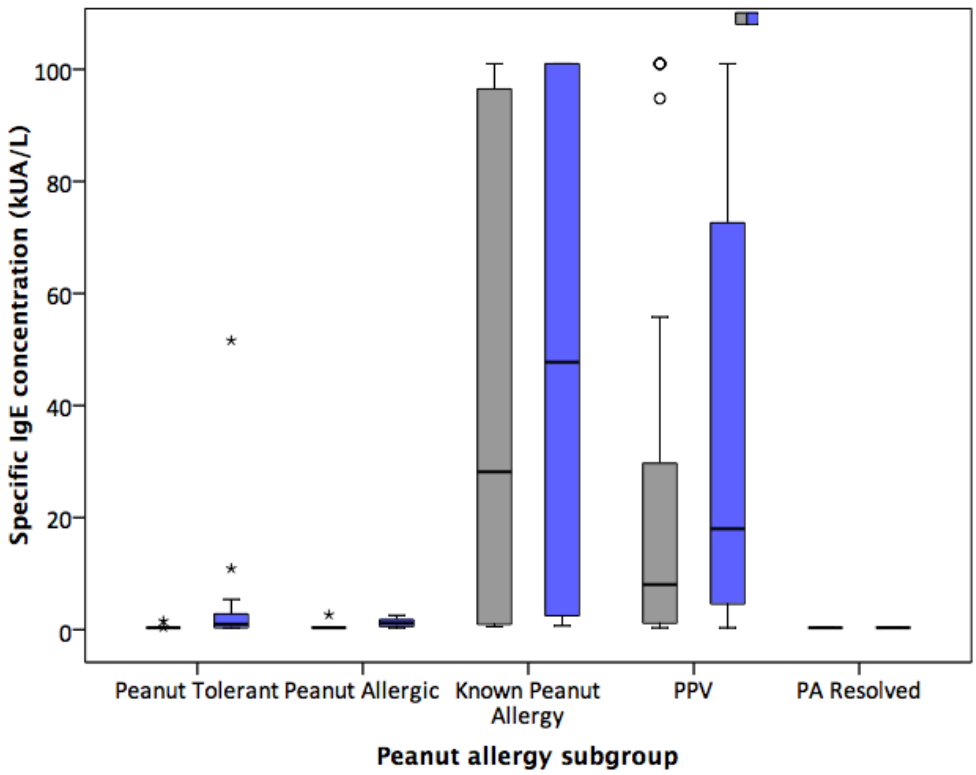
Ara h 2-specific IgE concentrations

A Kruskal-Wallis test identified a difference for at least one group (p<0.0001)(see Figure 16). Children with known peanut allergy and those with a test value above the positive predictive value had median Ara h 2-specific IgE concentrations of 28.2 kUA/L (IQR 97.9, 0.53-101) and 8.5 kUA/L (IQR 27.8, 0.34-101) respectively. Five post hoc Mann-Whitney U comparisons were made between children with known peanut allergy and challenge-proven peanut allergy (U=3.0,z=-3.130, p<0.005) and tolerance (U=3.0,z=-5.253, p<0.0001); positive predictive value and challenge-proven peanut allergy (U=53.5,z=-3.434, p<0.001) and tolerance (U=128.5,z=-6.368, p<0.0001) and, and children with challenge-proven peanut allergy and tolerance (U=91.0,z=-1.464, p>0.1). There was no significant difference when comparing median Ara h 2-specific IgE concentrations in de novo children with challenge proven allergy and peanut tolerant children; median 0.34kUA/L (IQR 0.12, 0.34-2.66) versus 0.34 kUA/L (IQR 0,0.34-1.54).

Comparison of whole peanut- and Ara h 2-specific IgE concentrations

Ara h 2-specific IgE concentrations tend to be lower than whole peanut-specific concentrations although the distribution of results was similar. Figure 16 compares whole peanut- and Ara h 2-specific IgE outcomes for the five subgroups. This highlights the significant difference between children in the positive predictive value and known peanut allergy subgroups and the other subgroups.

Figure 16: Whole peanut- and Ara h 2-specific IgE concentrations according to oral provocation challenge outcome subgroup



Legend:

Ara h 2-specific IgE
Whole peanut-specific IgE

Peanut allergic and tolerant children were further divided into subgroups; Challenge-proven peanut tolerance, challenge-proven peanut allergy, known peanut allergy, positive predictive value or resolved peanut allergy. Median whole peanut and Ara h 2 specific IgE values were highest for children with known peanut allergy and those in the positive predictive value subgroup (Kruskal-Wallis test; p<0.0001).

4.5.3 Summary of the comparison of peanut skin prick testing and whole peanut- and Ara h 2-specific IgE testing for the diagnosis of peanut allergy in egg-allergic, peanut-sensitised children

These analyses have demonstrated that all three tests are of limited value in distinguishing between peanut allergy and tolerance in peanut-naïve individuals whose test values lie within the immunological grey area below the published positive predictive value. For peanut skin prick testing, there was a difference in mean peanut skin prick test wheal diameter between the two groups, but this was only significant for the diagnosis of peanut allergy in children with a wheal diameter equal to or above the positive predictive value of 8mm. As there was no difference in the number of children with a positive skin prick test, then this test is only really useful in the identification of egg-allergic, peanut-naïve children who are at risk of peanut allergy. Subgroup analysis revealed results to be skewed by the inclusion of children with a wheal diameter above the previously published positive predictive value and those with

known peanut allergy. Skin prick testing was unhelpful if the differentiation of children with challenge-proven allergy or tolerance. However, a negative skin prick test is useful to screen out peanut tolerant children without the need for further investigation.

Analysis of both peanut- and Ara h 2-specific IgE testing identified a significant difference in the number of children with a positive test result between peanut allergic and peanut tolerant children but this was again skewed by children with known peanut allergy or whole peanut test values above the positive predictive value. The measurement of whole peanut-specific IgE concentrations and Ara h 2-specific IgE concentrations was, like skin testing, unable to discriminate between challenge-proven allergy and tolerance. This highlights the limited value of whole peanut-specific IgE concentrations in the diagnosis of peanut allergy in children with test values below the positive predictive value. There was a stronger association between having a positive Ara h 2-specific IgE concentration and peanut allergy, than having a positive whole-peanut specific IgE concentration and peanut allergy. A negative Ara h 2-specific IgE concentration increased the probability of tolerance but was insufficiently reliable and compared unfavourably with the peanut skin prick test for this purpose. Children with a negative Ara h 2-specific IgE concentration require further investigation in the form of an oral provocation challenge. All children with a positive Ara h 2-specific IgE concentration of Grade 3 or above were allergic.

4.6 Influence of the co-variables; persistent egg allergy, total IgE concentrations and age on oral provocation challenge outcomes

It is important to consider the presence of factors that may be associated with the prediction of either peanut allergy or peanut tolerance, as these may be valuable in the development of a diagnostic algorithm. It is also important to consider factors that might confound the analysis. Factors of potential interest identified in this study were the presence of persistent egg allergy, eczema, total IgE concentrations and the age range of included children.

4.6.1 Persistent egg allergy

54(77%) peanut allergic children had persistent egg allergy compared with 16 (52%) peanut tolerant children (Fisher's Exact, $p<0.05$). The presence of persistent egg allergy may be associated with an increased risk of a child being peanut allergic rather than peanut tolerant.

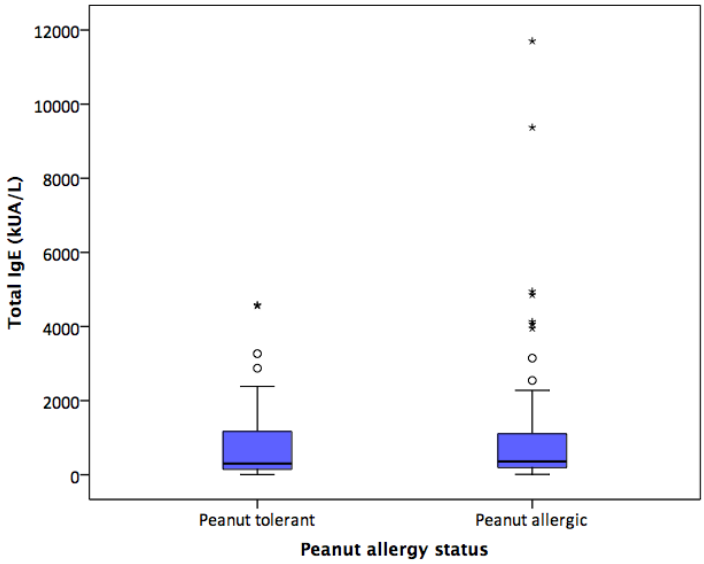
4.6.2 Eczema

All peanut allergic and peanut tolerant children had a history of current or previous eczema. The presence of eczema was not associated with an increased risk of a child being peanut allergic rather than peanut tolerant in this high-risk cohort of children with egg allergy.

4.6.3 Total IgE concentrations in allergic and tolerant children

It is possible that peanut- and Ara h 2-specific IgE values may have been affected by differences in total IgE concentrations between peanut allergic and peanut tolerant children. Therefore, total IgE concentrations were compared between these two groups but no difference was found. Figure 18 shows total IgE concentrations for children in the peanut allergic and tolerant groups. The median total IgE was 359 kUA/L (IQR 932, 11-11702) in children with peanut allergy and 302 kUA/L (IQR 1054, 6-4584) in peanut tolerant children, showing no difference between the two groups (Figure 17).

Figure 17. Total IgE concentrations in peanut allergic and tolerant children



Legend: The study population was divided into two primary outcome groups; those who were peanut allergic and those who were peanut tolerant. There was no difference in total IgE concentration between allergic and tolerant children (p>0.05).

4.6.4 Analysis of peanut skin prick tests and the measurement of whole peanut- and Ara h 2-specific IgE concentrations when study children are categorised according to age

Differences in food-specific IgE concentrations have been described for children aged below and over two years of age as described above and the ages of subjects in this study has been examined accordingly. The majority of children were older than two years of age. 14(14%) children were less than two years of age and 87(86%) were two years of age or older. The

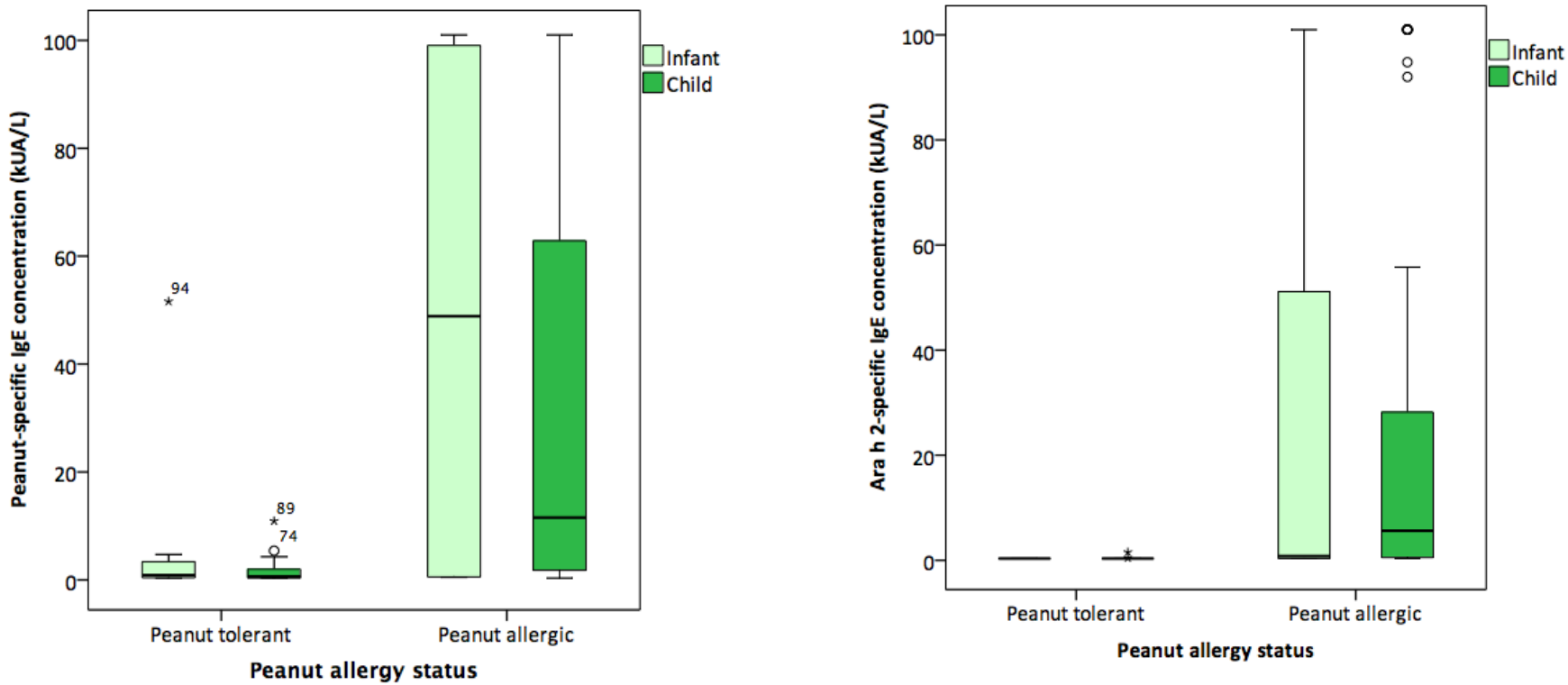
sample of infants was small and therefore it is difficult to analyse the available data. Among the infants, no peanut tolerant child had a positive Ara h 2-specific IgE concentration although the tolerant child with a high whole peanut-specific IgE of Grade 5 was an infant. Most tolerant children also had low Ara h 2 grades but many allergic children also had low grade or negative whole peanut- and Ara h 2-specific IgE concentrations.

Figure 18 depicts graded whole peanut- and Ara h 2-specific IgE classifications separately for infants and children. Children were not found to have higher median specific IgE values than infants as has often been previously reported. Peanut allergic infants had median whole peanut-specific IgE concentrations of 48.9 kUA/L (IQR 100, 0-101) compared with allergic children 11.6 kUA/L (IQR 63, 0-101). Peanut tolerant children had median whole peanut-specific IgE concentrations of 0.5 kUA/L (IQR 2, 0-11) compared with tolerant infants 0.9 kUA/L (IQR 4, 0-52). Median Ara h 2-specific IgE concentrations were lower than whole peanut-specific IgE concentrations for allergic and tolerant infants and children. Peanut allergic infants had median Ara h 2-specific IgE concentrations of 0.8 kUA/L (IQR 76, 0-101) compared with allergic children 5.6 kUA/L (IQR 28, 0-101). Both peanut tolerant infants and children had median Ara h 2-specific IgE concentrations of 0.34 kUA/L.

4.6.5 Conclusion of analysis of co-variables on oral provocation challenge outcome

There was no discernible difference in test results between infants and children. As the sample size of infants was smaller than anticipated, no further analyses have been completed separating children by age. Total IgE concentrations also had no effect upon peanut allergy status. There was an association between persistent egg allergy and a diagnosis of peanut allergy, and this will be considered during logistic regression modelling in Section 4.7.

Figure 18: Differences between whole peanut and Ara h 2-specific IgE concentrations in allergic and tolerant infants and children



Legend: The study population was divided into primary outcome groups; those who were peanut allergic and those who were peanut tolerant. Children were not found to have higher median specific IgE values than infants. Children less than two years of age were classified as infants.

4.7 Logistic Regression to predict the probability of peanut allergy being detected by peanut skin prick testing, or whole peanut- or Ara h 2-specific IgE concentrations

Logistic regression is applicable to a continuous measurement (food-specific IgE antibody concentration) and the nominal categorical (binary) variable of being peanut allergic or tolerant. Logistic regression enables the prediction of the probability of the nominal variable (peanut allergy status) being based on the independent variables. This was performed to predict the probability of peanut allergy being detected by either a peanut skin prick test, or whole peanut- or Ara h 2-specific IgE concentration. Logistic regression was performed independently for each of the three tests and for persistent egg allergy. Results are shown in Table 16.

Table 16: Logistic regression predicting likelihood of peanut allergy based on measurement of peanut SPT wheal diameter and whole peanut- and Ara h 2-specific IgE concentrations

Test	*B* (SE)	*p*	Odds ratio	95% CI for Odds Ratio	
				Lower	Upper
Ara h 2-Specific IgE	2.90 (1.32)	0.03	18.3	1.37	245.4
Peanut-Specific IgE	0.08 (0.34)	0.02	1.09	1.01	1.16
Peanut-Skin prick test	0.51 (0.11)	<0.0001	1.67	1.34	2.06
Persistent egg allergy	1.15 (0.46)	0.01	3.16	1.29	7.77

Legend: Persistent egg allergy was included in the model as it had been identified as being associated with a diagnosis of peanut allergy in Section 4.6

The logistic regression model based on a positive Ara h 2-specific IgE concentration of ≥0.35 kUA/L was statistically significant, p<0.0005. Based on the Nagelkerke *R2*, the model only explained 57% of the variance in peanut allergy with the other 43% of factors being unidentified. Children with a positive Ara h 2-specific IgE concentration had an odds ratio of 18. Therefore the risk of being peanut allergic increased by 18 for each unit change in Ara h 2-specific IgE which was statistically significant despite having wide 95% confidence intervals. Given these wide margins of error, the precision of the measurement of Ara h 2-specific IgE concentrations is low. The logistic regression model for whole peanut-specific IgE and a positive peanut skin prick test had lower odds ratios of 1.085 and 1.665 respectively but also have far smaller 95% confidence intervals meaning that these tests are more precise. Skin prick test wheal diameters to peanut were indicated to be an important factor (p<0.05) with the independent odds ratio of being peanut allergic increasing by 1.7 for each mm increase in wheal diameter with the 95% confidence interval ranging from 1.34 to 2.06. The logistic regression model for persistent egg allergy similarly demonstrated a lower odds ratio than for

a positive Ara h 2-specific IgE concentration, being 3.164 (95% CI: 1.288-7.771), although the 95% confidence intervals here were slightly wider reflecting decreased precision.

A series of logistic regression models have all demonstrated a significant relationship between either a positive skin prick test to peanut, a positive whole peanut-specific IgE level or a positive Ara h 2-specific IgE level and peanut allergy although the extent to which these can be relied upon varies due to their varying degrees of statistical significance. Logistic regression modelling confirms the significant relationship between a child having persistent egg allergy and peanut allergy.

4.8 Likelihood Ratios to examine the clinical utility of peanut skin prick testing, whole peanut- and Ara h 2-specific IgE measurements in predicting peanut allergy in egg-allergic peanut-sensitised children

Sensitivity and specificity are not helpful in practical terms but can be combined to produce a likelihood ratio that is useful when reviewing an individual patient. Table 18 shows likelihood ratios for both the two groups of peanut allergic and tolerant children and the further subgroups.

4.8.1 Likelihood ratios for two groups of peanut allergic and peanut tolerant children

The pre-test probability of a child chosen at random from a group of egg-allergic children being reviewed in the tertiary paediatric allergy clinic having peanut allergy is 0.69 (70/101). The specificity and sensitivity for the three tests: peanut skin prick test, whole peanut-specific IgE and Ara h 2-specific IgE were calculated using the manufacturer's recommended cut-off values using this pre-test probability. Sensitivity for Ara h 2-specific IgE testing was inferior to that of both whole peanut-specific IgE at 94% and skin prick testing at 96%. A positive Ara h 2-specific IgE concentration had a 97% positive predictive value for the diagnosis of peanut allergy with a negative predictive value of 69%. Ara h 2-specific IgE concentrations had the highest likelihood ratio with highest post-test odds of having peanut allergy with a positive Ara h 2-specific IgE concentration result being 97% (Table 18). The Fagan's nomogram used to calculate post-test odds is included in Appendix 8.

4.8.2 Likelihood ratios calculated for subgroups of peanut allergic and tolerant children

Children subjected to an oral provocation challenge had a pre-test probability of 0.21 (8/36) for having peanut allergy. In this analysis of children with challenge-proven allergy or tolerance, the sensitivity for Ara h 2-specific IgE concentrations falls notably from the previous analysis of two groups of allergic and tolerant children (Table 18). Specificity remains highest for Ara h 2-specific IgE concentrations at 94%. The positive predictive values for all tests are poor. The negative predictive values performed better in this analysis. Ara h 2-specific IgE concentrations still produced the highest positive likelihood ratio of 5.81 and had the highest post-test odds of 60%. In this subgroup analysis, a child with positive Ara h 2-specific IgE has an increased probability of having peanut allergy raised from the pre-test probability of 21% to post-test probability of 60%.

The pre-test probability for children with known allergy was also 0.21 (8/36) (Table 18). All three tests had a sensitivity of 100%. Specificity was similar to that for challenged children, being highest for the measurement of Ara h 2-specific IgE at 94%. The positive predictive value

was also highest for a positive Ara h 2-specific IgE concentration at 80% and all three tests had a 100% negative predictive value. Ara h 2-specific IgE had the highest positive likelihood ratio of 15.5 compared with values just below 1.5 for both peanut tests. Post-test odds remained highest for Ara h 2-specific IgE concentrations. A child with a positive Ara h 2-specific IgE concentration had an increased probability of peanut allergy, raised from the pre-test probability of 21% to post-test probability of 79%.

The pre-test probability in the subgroup of children with whole peanut-specific IgE or skin prick tests above previously published 95% positive predictive values was 0.64 (Table 18). Sensitivity was good for all three tests but specificity was better for Ara h 2-specfic IgE concentrations at 94%. Again, Ara h 2-specific IgE concentrations yielded the highest positive predictive value at 96%. All three groups had comparable negative predictive values. The highest positive likelihood ratio was for Ara h 2-specific IgE testing at 13.2. A child with positive Ara h 2-specific IgE in this subgroup has an increased probability of having peanut allergy raised from the pre-test probability of 64% to post-test probability of 96%.

4.8.3 Summary of likelihood ratio analysis

In summary, out of all three available tests, the measurement of Ara h 2-specific IgE concentrations had the best clinical utility (Table 18). A child with positive Ara h 2-specific IgE has an increased probability of having peanut allergy raised from the pre-test probability of 69% to post-test probability of 97%. For all analyses, including subgroup analyses, a positive Ara h 2-specific IgE concentration increased the post-test odds of a child having peanut allergy to a greater extent than for either of the other two tests. Sensitivity tended to be higher for whole peanut-specific IgE and skin prick tests although Ara h 2-specific IgE testing had the best specificity in the majority of the above analyses. It is important to consider where the balance between sensitivity and specificity should lie to achieve optimum clinical utility when selecting a diagnostic test. This will be taken into consideration when constructing receiver-operator characteristic curves in Section 4.9.

Table 17: Sensitivity, specificity, positive predictive values and negative predictive values for cohorts of peanut allergic and tolerant children

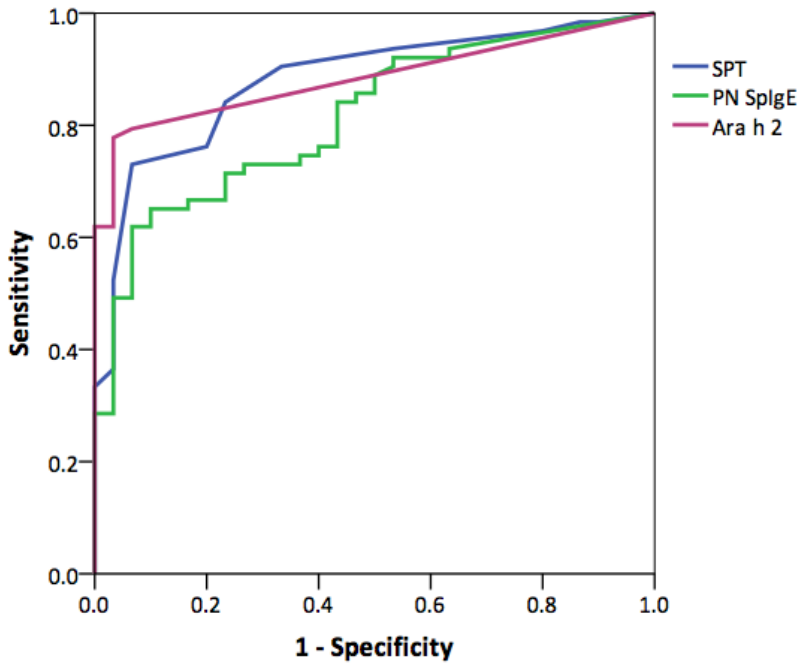
Test	Analysis cohort	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Positive Likelihood Ratio	Negative Likelihood Ratio	Post-test probability	
								+ Test	-Test
Ara h 2-specific IgE	Full study population	81%	94%	97%	69%	12.62	0.20	97%	31%
Whole peanut-specific IgE	Full study population	94%	32%	75%	71%	1.39	0.18	75%	28%
Peanut skin prick test	Full study population	96%	23%	74%	70%	1.25	0.19	74%	30%
Ara h 2-specific IgE	Challenge-proven population	38%	94%	60%	85%	5.81	0.67	60%	15%
Whole peanut-specific IgE	Challenge-proven population	88%	32%	25%	91%	1.29	0.39	25%	9%
Peanut skin prick test	Challenge-proven population	88%	23%	23%	88%	1.14	0.54	23%	13%
Ara h 2-specific IgE	Positive predictive value	85%	94%	96%	78%	13.2	0.16	96%	22%
Whole peanut-specific IgE	Positive predictive value	94%	32%	70%	77%	1.39	0.18	70%	23%
Peanut skin prick test	Positive predictive value	96%	23%	69%	78%	1.26	0.16	69%	22%

Legend: Sensitivity and specificity are not helpful in practical terms but can be combined to produce a likelihood ratio. A positive likelihood ratio of 1 means that a positive test is more likely to occur in a child with peanut allergy rather than a tolerant child. Likelihood ratios can be used in conjunction with the pre-test probability of a child having peanut allergy to calculate their post test probability of being peanut allergic

4.9 Receiver Operator Characteristic Curves to compare the accuracy of the three diagnostic tests; the peanut skin prick test and the measurement of whole peanut- and Ara h 2-specific IgE concentrations

The receiver operating characteristic (ROC) curve is obtained by plotting the sensitivity of a test against 1-specificity. The area under the ROC curve provides a measure by which to compare the accuracy of diagnostic tests (Akobeng, 2007). Receiver operator curves (ROC) were constructed to compare the diagnostic utility of each test: peanut skin prick test wheal diameters, whole peanut-specific IgE concentrations and Ara h 2-specific IgE concentrations (Figure 19).

Figure 19: Receiver-operator characteristic curves showing the performance of the three screening tests in children with peanut allergy and tolerance in predicting peanut allergy



Legend. The ROC curve is obtained by calculating the sensitivity and specificity of a test at every possible cut-off point, and plotting sensitivity against 1-specificity. An area under the curve of greater than 80% suggests that the test is a good diagnostic test with clinical utility. In practice this means that if a clinician were to take two children, one allergic and one tolerant and perform the selected test on them, then the child with the abnormal test result should be the child from the peanut allergic group. Ara h 2 is the component with the highest accuracy for discriminating between peanut allergy or tolerance.

The area under the curve calculations are presented in Table 19. The receiver-operator characteristic curve for Ara h 2-specific IgE concentrations revealed an area under the curve of 0.88 (95% CI 0.88-0.95) ($p<0.0005$). This compares with other studies; Kim reported an area under the curve of 82% and a recent systematic review identified a range of area under the curve values for Ara h 2-specific IgE of between 0.90 and 0.99 (Klemans, 2015, Eller and Bindslev-Jensen, 2013). However, the other tests also both had equivalent clinical utility.

Whole peanut-specific IgE had an area under the curve of 0.82 (95% CI 0.73-0.90) (p<0.0005) and the peanut skin prick test had an area under the curve of 0.88 (95% CI 0.82-0.95) (p<0.0005). Therefore although Ara h 2-specific IgE was demonstrated to have the best diagnostic accuracy for the prediction of clinical peanut allergy the area under the curve was not significantly different to the other two tests.

Table 18: Area under the curve for skin prick test to peanut, whole peanut-specific IgE concentrations and Ara h 2-specific IgE concentrations

Test	AUC [95% CI)	*p* value
Ara h 2-specific IgE	0.88 [0.81-0.95]	<0.0005
Peanut-specific IgE	0.81 [0.73-0.90]	<0.0005
Peanut skin prick test	0.88 [0.82-0.95]	<0.0005

Legend. Ara h 2 was demonstrated to have the best diagnostic accuracy for the prediction of peanut allergy but values were similar for all three tests

4.9.1 The selection of optimal cut-off values from the receiver operator characteristic curve for the prediction of peanut allergy in the study population

The selection of optimal cut-off values for a diagnostic test depends upon the desired balance between sensitivity and specificity. Several cut-off values are examined below selected by utilising the following in turn: (1) the Youden Index, (2) the manufacturer's cut-off value, (3) previously published 95% positive predictive values, (4) those giving the highest sensitivity, (5) those giving the highest specificity and (6) those where both sensitivity and specificity were as close to 80% as possible (Table 20).

The selection of cut-off values using the Youden Index assumes that both false positive and false negative diagnoses are equally undesirable. Although these may be the best fit cut-off values identified from the receiver operator characteristic curve, they have reduced utility in the clinical setting due to the risk of leading to more false negative results than is acceptable. The manufacturer's cut-off values were analysed, as it is important to establish the applicability of the manufacturer's cut-off value to the study population. General practitioners who have access to specific IgE laboratory testing often base their decision on these cut-off values. Cut-off values calculated using previously published 95% positive predictive values were also examined. The 8mm cut-off value for peanut skin prick test performed better than whole peanut-specific IgE testing, having 73% sensitivity and 90% specificity. Sampson's 95% positive predictive value for whole peanut-specific IgE had good specificity at 97% but surprisingly poor sensitivity in this study population at 46%. Those cut-off values that yielded

the highest sensitivity were identified followed by those that produced the highest specificity. These results demonstrated giving priority to one test to be at the expense of the other, although Ara h 2-specific IgE concentrations offered the best performance confirmed by the highest Youden Index. False negatives would place the child at risk of experiencing a potentially severe reaction in an unsafe environment. Therefore a test with good sensitivity at the expense of good specificity is far from ideal. Specificity is of prime importance. It is more desirable to have more children with false positive tests as opposed to false negative tests for safety reasons. The ideal scenario is to establish a test that eliminates the need for an oral provocation challenge.

A secondary aim of the study was to identify optimal decision points. The identification of cut-off values close to 80% is an attempt to achieve this balance. Ara h 2-specific IgE testing had a low optimal cut-off value of 0.39 kUA/L with sensitivity of 79% and good specificity of 93%. The present study did not find Ara h 2 to be as good a test as has been previously reported. A study of UK schoolchildren reported a similar cut-off value of 0.35kUA/L to classify all peanut allergic children correctly, with 100% sensitivity and 96% specificity (Nicolaou et al., 2011). However, the study population in the latter study had originated from a birth cohort study. In the present study, cut-off values for optimal peanut skin prick test wheal diameters and specific IgE concentrations were higher than for Ara h 2-specific IgE concentrations. For whole peanut-specific IgE a cut-off value of 1.08 kUA/L gave 81% sensitivity although at the expense of 57% specificity. To achieve a specificity of 80%, the cut-off value needed to be raised to 3.36 kUA/L although corresponding sensitivity fell to 67%. For peanut skin prick testing, a 6mm wheal diameter gave reasonable sensitivity and specificity of 84% and 80% respectively.

4.9.2 Conclusion of the construction of receiver operator characteristic curves for the comparison of diagnostic accuracy and the identification of optimal cut-off values for the three tests

In summary, although all three tests have a similar area under the curve, when considering the balance of sensitivity and specificity, the measurement of Ara h 2-specific IgE concentrations had the best performance for all of the proposed cut-off values presented above. The optimal decision points identified in this analysis will be used in Chapter 5 to construct a model proposing a diagnostic algorithm.

Table 19: Area under the curve and optimal cut-off values for the diagnosis of peanut allergy constructed for skin prick test to peanut, whole peanut-specific IgE concentrations and Ara h 2-specific IgE concentrations based on the Youden Index

Test	Factors determining the selected cut-off value	Selected Cut-off value	Sensitivity	Specificity	Youden Index
Ara h 2-specific IgE	Youden Index	0.45 kUA/L	78%	97%	0.74
Whole peanut-specific IgE	Youden Index	5.99 kUA/L	62%	93%	0.46
Peanut skin prick test	Youden Index	7.5 mm	73%	90%	0.66
Ara h 2-specific IgE	Manufacturer's cut-off values	0.35 kUA/L	79%	93%	0.73
Whole peanut-specific IgE	Manufacturer's cut-off values	0.35 kUA/L	94%	37%	0.30
Peanut skin prick test	Manufacturer's cut-off values	3 mm	94%	50%	0.40
Whole peanut-specific IgE	95% positive predictive value	15 kUA/L	46%	97%	0.43
Peanut skin prick test	95% positive predictive value	8mm	73%	90%	0.66
Ara h 2-specific IgE	Priority to sensitivity	0.39 kUA/L	79%	93%	0.73
Ara h 2-specific IgE	Priority to specificity	1.77 kUA/L	62%	100%	0.62
Whole peanut-specific IgE	Priority to sensitivity	0.35 kUA/L	94%	37%	0.30
Whole peanut-specific IgE	Priority to specificity	54.96 kUA/L	29%	100%	0.29
Peanut skin prick test	Priority to sensitivity	3 mm	94%	50%	0.40
Peanut skin prick test	Priority to specificity	10 mm	37%	100%	0.33
Ara h 2-specific IgE	Optimal decision point	0.39 kUA/L*	79%	93%	0.73
Whole peanut-specific IgE	Optimal decision point	1.08 kUA/L*	81%	57%	0.38
Whole peanut-specific IgE	Optimal decision point	3.36 kUA/L*	67%	80%	0.47
Peanut skin prick test	Optimal decision point	6mm*	84%	80%	0.61

Legend. There is no 95% positive predictive value available for Ara h 2-specific IgE concentrations. Cut-off values were selected based upon the best balance of sensitivity and specificity, considering the clinical need for safety. This balance leans towards a false positive test being preferable to a false negative test.

Chapter 5

Results Section 2: Data interpretation and application

Peanut vendor, Bangkok 2015

Peanut vendors are still common today in Asia.
This image is in the public domain
https://www.flickr.com/photos/tordremme/23660466425

CHAPTER 5

RESULTS SECTION 2: DATA INTERPRETATION AND APPLICATION

5.1 Introduction

The secondary aim of this study was to evaluate whether the measurement of Ara h 2-specific IgE concentrations is clinically useful in the management of peanut-naïve, peanut-sensitised children with a history of egg allergy, and to construct optimal cut-off values for the three examined tests: peanut skin prick testing, whole peanut-specific IgE concentrations and Ara h 2-specific IgE concentrations. An ideal cut-off value for the diagnosis of peanut allergy in egg-allergic, peanut-sensitised children is one that will reduce the need for a child to be subjected to an oral provocation challenge. Current testing using peanut skin prick tests and/or whole peanut-specific IgE concentrations leaves a large number of children with test values below previously published positive predictive values sitting within the immunological grey area. Given that the option of leaving a child without a definitive diagnosis is no longer acceptable, if indeed it ever has been, this population of children require an oral provocation challenge which is time-consuming, labour-intensive, costly, and stressful for children and families. Optimal clinical decision points were identified from the ROC curve analysis for the three tests by selecting cut-off values that had both sensitivity and specificity as close to 80% as possible, as discussed in Chapter 4. Using these criteria the best cut-off values were: ≥6mm for peanut skin prick testing, ≥1.08kUA/L for whole peanut-specific IgE testing and ≥0.39kUA/L for Ara h 2-specific IgE.

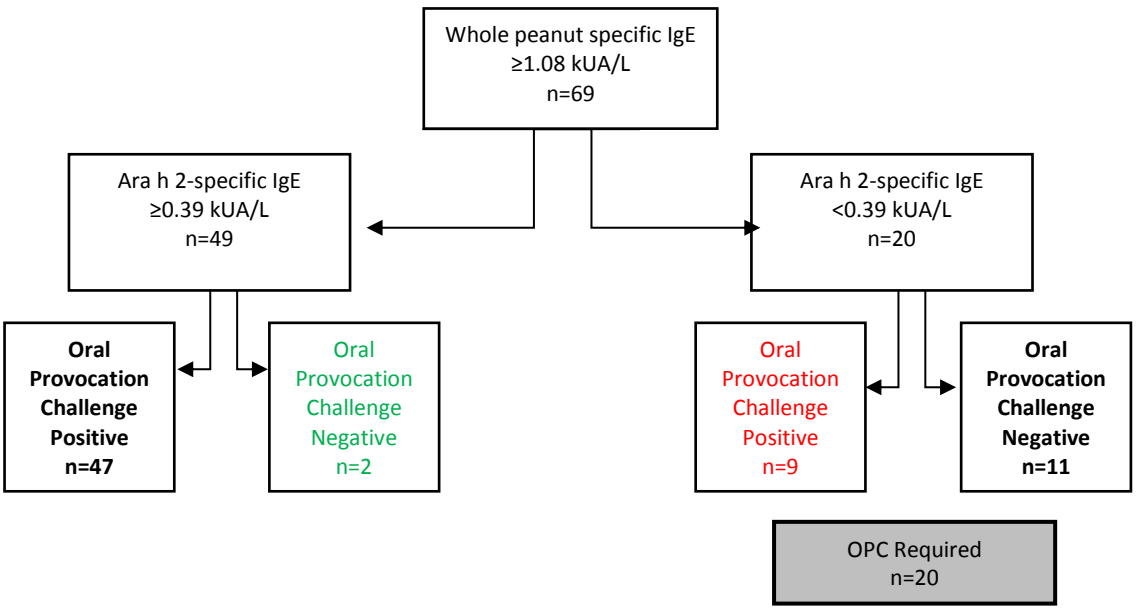
5.2 Examination of a potential stepwise approach diagnostic algorithm

Examination of the clinical utility of whole peanut- and Ara h 2-specific IgE concentrations and peanut skin prick tests has highlighted a high diagnostic error rate. The measurement of Ara h 2-specific IgE concentrations was superior to both whole peanut-specific IgE and peanut skin prick testing in the present study but their use as a replacement for the gold standard oral provocation challenge is limited. Ara h 2-specific IgE testing is not yet at the stage where it can be used to replace the oral provocation challenge. Using the identified cut-off values, an attempt was made to identify a stepwise approach to identify those children most at risk of peanut allergy and to subsequently reduce the number of children requiring a peanut provocation challenge. 4 separate models are examined.

5.2.1 Model 1: Whole peanut-specific IgE concentration testing followed by Ara h 2-specific IgE concentration testing

The first model approach examined a two-step approach commencing with a whole peanut-specific IgE concentration utilising a cut-off of 1.08 kUA/L and identified 69 children from the entire cohort (Figure 20). Children without whole peanut-specific IgE measurements available were excluded. This model assumes that children with a whole peanut-specific IgE concentration below 1.08 kUA/L would be peanut tolerant and would have led to 9 (13%) peanut allergic children being misclassified as peanut tolerant and 11 (16%) being correctly classified as tolerant. For the second step of the algorithm, the 20 children with an Ara h 2-specific IgE concentration of less than 0.39 were then excluded and presumed tolerant. This led to 11 (16%) children being correctly classified as tolerant and 9 (13%) peanut allergic children being misclassified as peanut tolerant, which is clearly a potentially dangerous situation. The remaining 49 (71%) children all had an Ara h 2-specific IgE concentration of 0.39 or above, the presumed allergic group. Within this group, 47 (68%) children were correctly classified as peanut allergic whilst only 2 peanut tolerant children were misclassified as allergic. This is an acceptable misclassification rate as it does not pose a risk to children and the rate of being falsely labelled peanut allergic is not very high. Overall, Model 1 misclassified 11 children. Children with an Ara h 2-specific IgE concentration below the 0.39kUA/L cut-off value need to be challenged rather than being labelled as tolerant but overall, use of this model reduces the number of oral provocation challenges from 69 to 20.

Figure 20: Model 1: A two-step diagnostic algorithm utilising whole peanut-specific IgE concentrations followed by Ara h 2-specific IgE concentrations



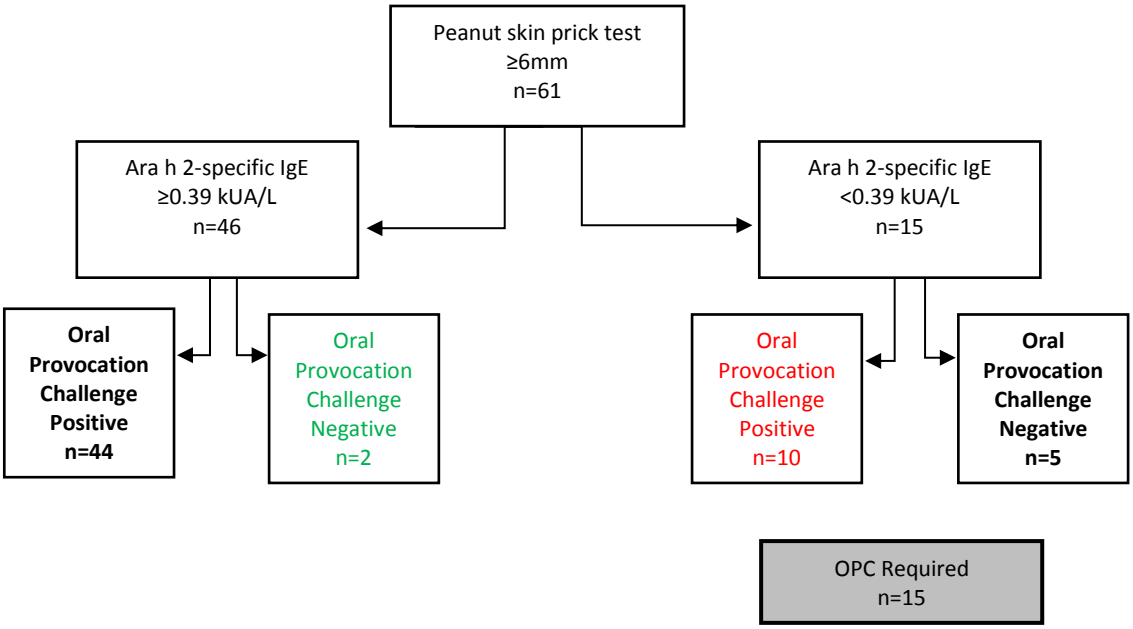
Legend. Acceptable misclassified children appear in green; unacceptable misclassified children appear in red. Outcomes in black and bold are correctly classified.

A similar approach of measuring whole peanut-specific IgE concentrations followed by Ara h 2-specific IgE concentrations was undertaken within the HealthNuts birth cohort population and was reported to reduce the number of children requiring a confirmatory oral provocation challenge by two thirds (Dang et al., 2012). The HealthNuts study identified a 15kUA/L positive predictive value for whole peanut-specific IgE to have a corresponding specificity of 98% and a sensitivity of 26%. The current study identified a better performance with whole peanut-specific IgE concentrations having 97% specificity and 46% sensitivity. At the equivalent specificity of 98%, the current study identified the sensitivity of Ara h 2-specific IgE testing to be 77%, compared with 60% within the HealthNuts study. Therefore, Ara h 2-specific IgE concentrations correctly identify a higher proportion of children with peanut allergy than whole peanut-specific IgE.

5.2.2 Model 2: Skin prick testing followed by Ara h 2-specific IgE concentration testing

In model 2, the initial step of measuring whole peanut-specific IgE concentrations was replaced by a skin prick test utilising a cut-off value of ≥6mm. According to this model, children with a wheal diameter below 6mm would be considered peanut tolerant. (A possible strategy for the management of these children is discussed later in Chapter 6). Children on antihistamines who did not undergo skin testing were excluded. 61 children were identified (Figure 21). For the second step of the algorithm, the 15 children with an Ara h 2-specific IgE concentration of less than 0.39 kUA/L were then excluded and presumed tolerant. This led to 10 children being correctly classified as tolerant and 5 peanut allergic children being misclassified as peanut tolerant, which like Model 1 again does not yield an acceptable misclassification rate. The remaining 46 children all had an Ara h 2-specific IgE concentration of 0.39 or above and comprise the presumed allergic group. Within this group, 44 children were correctly classified as peanut allergic whilst only 2 peanut tolerant children were misclassified as allergic which is a similar acceptable misclassification rate to that above in Model 1. Overall, Model 2 misclassified 12 children with 10 (13%) being unacceptably misclassified. As above in Model 1, children with an Ara h 2-specific IgE concentration below the 0.39kUA/L cut-off value need to be challenged. In the study population, 15 children would have required an oral provocation challenge under this model. Model 2 is potentially more useful than Model 1, as the skin prick test is a quicker and easier screening method and due to its high negative predictive value, enables the clinician to safely eliminate peanut allergy in a large number of egg-allergic children without the need for venepuncture or other further investigation.

Figure 21: Model 2 - A two-step diagnostic algorithm utilising peanut skin prick test followed by Ara h 2-specific IgE concentration



Legend. Acceptable misclassified children appear in green; unacceptable misclassified children appear in red. Outcomes in black and bold are correctly classified.

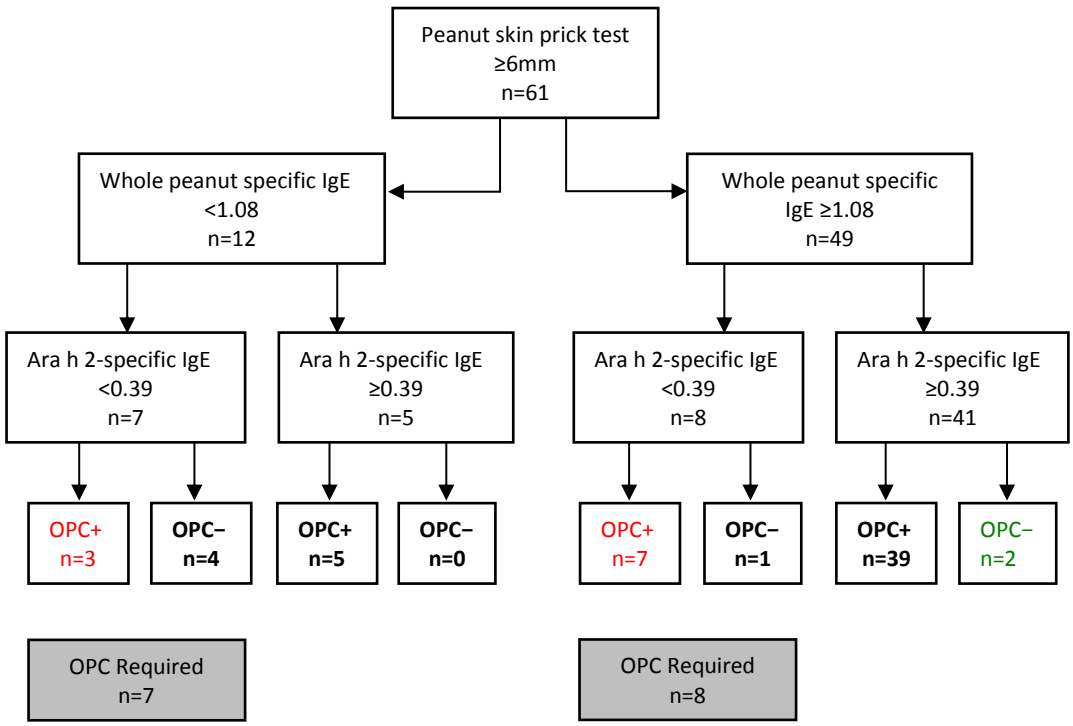
Neither Model 1 nor Model 2 were good at classifying tolerance in children although did have reasonable classification rates for diagnosing peanut allergy which will reduce the immunological grey area. An improved method of confirming peanut tolerance for children with positive whole peanut-specific IgE concentrations or peanut skin prick test wheal diameters is needed to eliminate the need for a peanut oral provocation challenge. However, this two-step stepwise approach appears to have clinical utility for the diagnosis of peanut allergy in egg-allergic, peanut-sensitised children.

5.2.3 Model 3: Peanut skin prick testing followed by whole peanut-specific IgE concentration testing followed by Ara h 2-specific IgE concentration testing

A further stepwise approach model was constructed to add in a third diagnostic step (Figure 22). Skin prick test was used as the initial screening measure, being the easiest to perform in the majority of children attending clinic. Whole peanut-specific IgE concentrations were then added in as the second step to create two subgroups of children; those with whole peanut-specific IgE concentrations (a) below and (b) above the 1.08 kUA/L cut-off value. 49(80%) children had test results of ≥1.08 kUA/L and 12(20%) children had values less than this cut-off. The third step was to add in Ara h 2-specific IgE concentrations ≥0.39 kUA/L for the analysis of children in both whole peanut-specific IgE subgroups. This created four further subgroups; children with (A) whole peanut ≥1.08 kUA/L and Ara h 2-specific IgE <0.39 kUA/L, (B) whole

peanut ≥1.08 kUA/L and Ara h 2-specific IgE ≥0.39 kUA/L, (C) whole peanut-specific IgE <1.08 kUA/L and Ara h 2-specific IgE ≥0.39 kUA/L, and (D) whole peanut-specific IgE <1.08 and Ara h 2-specific IgE <0.39 kUA/L.

Figure 22: Model 3 - A stepwise approach for the diagnosis of peanut allergy using study generated cut-off values for peanut skin prick testing, whole peanut- and Ara h 2-specific IgE concentrations



***Legend. Acceptable misclassified children appear in green; unacceptable misclassified children appear in red. Outcomes in black and bold are correctly classified. OPC,* oral provocation challenge.**

Among children with a negative whole peanut-specific IgE and a positive Ara h 2-specific IgE concentration (C), all 5 (9%) children were classified correctly as peanut allergic. The next best performance was for children with both a positive whole peanut- and a positive Ara h 2-specific IgE concentration (B) which correctly identified 39 (64%) allergic children and misclassified only 2 (3%) children as peanut-allergic rather than peanut-tolerant. This misclassification would be acceptable in clinical practice as it does not place any children at risk and does not give a positive diagnosis to many children. 3 (5%) children with negative test results to both tests (D) were misclassified as tolerant when they were allergic. 7 (11%) of allergic children with a positive peanut but a negative Ara h 2-specific IgE concentration (A) and were misclassified as tolerant.

Overall, 12 (20%) children were misclassified when following Model 3 which is comparable to the misclassification rates for both earlier models. The classification profile was similar to the above models with positive Ara h 2-specific IgE concentrations performing well in children with a peanut skin prick test wheal diameter of ≥6mm, regardless of whole peanut-specific IgE concentration. Again, negative Ara h 2-specific IgE concentrations were unreliable and placed children at potential risk. This suggests that there is no advantage to the more complex and expensive three-step model.

5.2.4 Model 4: Children with a peanut screening skin prick test wheal diameter <6mm

Children with a skin prick test wheal diameter of <6mm are not included in the above stepwise models and require further evaluation via a separate stepwise approach (Model 4). 10 peanut allergic children within the study fell into this category; 5 with challenge-proven peanut allergy, 2 with known peanut allergy and 3 with a whole peanut-specific IgE of ≥15kUA/L. As most children with a whole peanut-specific IgE concentration above 15 kUA/L are routinely excluded for oral provocation challenges for safety reasons, this leaves 7 of the 10 children requiring further investigation. 23 peanut tolerant children also had a skin prick test diameter of less than 6mm; 3 of whom had an entirely negative test, leaving 21 tolerant children requiring further investigation.

A total of 27 (28%) children would fall into an immunological grey area by virtue of their low skin prick test diameters. Most of these children proved tolerant but it is not easy to discriminate between allergic and tolerant children, meaning that most of them will require further investigation. As discussed earlier, the performance of the three tests between children with challenge-proven peanut allergy and challenge proven tolerance was unhelpful.

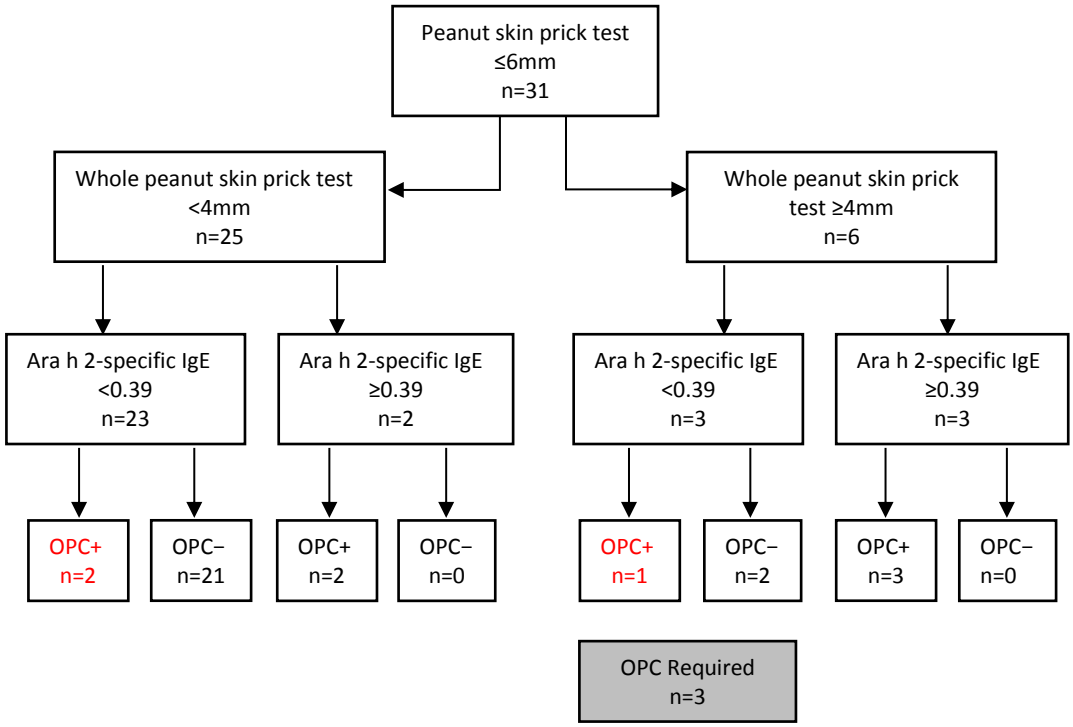
Close scrutiny of the data revealed that if children with a peanut skin prick test wheal diameter of <4mm were labelled as peanut tolerant, then 21 would be correctly identified as tolerant. (These children also had a negative Ara h 2-specific-IgE concentration <0.39kUA/L). Challenging children with a negative skin prick test of <4mm and a negative Ara h 2-specific IgE concentration would result in the need for 23 challenges, which places a significant drain upon hospital resources given that in this cohort of children only 2 of the 23 challenged children reacted. Therefore the model needs to be further refined.

If a 4mm cut-off value for skin prick testing were to be applied to the current cohort of peanut allergic children, then 3 children would be misclassified as being tolerant. This could be refined

by including an Ara h 2-specific IgE <0.39 kUA/L cut-off value as a second step, as in Model 4. Utilising this model, only 2 allergic children with both skin prick tests wheal diameters to whole peanut and negative Ara h 2-specfic IgE concentrations would be then be misclassified as peanut tolerant (Figure 23). Children with an Ara h 2-specific IgE concentration above 0.39 kUA/L in this Model could be diagnosed as peanut allergic without the need for an oral provocation challenge. Model 4 does reduce the number of children who would require a peanut oral provocation challenge down to 3 - these being children with a skin prick test to peanut greater than 4mm but a negative Ara h 2-specific IgE concentration - but did also place 2 children at risk given that 2 children having both a skin prick test to whole peanut below 4mm and an Ara h 2-specific IgE concentration of less than 0.39 kUA/L reacted upon peanut oral provocation challenge.

Model 4 demonstrates that most peanut allergic children continued to have a positive Ara h 2-specific IgE concentration ≥0.39kUA/L. All 5 children with a positive Ara h 2-specific IgE concentration were peanut allergic so this group of children may not need to be challenged. Three children with a negative Ara h 2-specific IgE concentration proved allergic; 1 of these had a skin prick test ≥4mm.

Figure 23: Model 4: A two-step diagnostic algorithm for the evaluation of peanut-sensitised children with a peanut skin prick test wheal diameter ≤6mm



***Legend. Acceptable misclassified children appear in green; unacceptable misclassified children appear in red. Outcomes in black and bold are correctly classified. OPC,* oral provocation challenge.**

5.3 Summary of stepwise approach

Neither of the existing widely used whole peanut-specific IgE and skin prick tests, nor the Ara h 2-specific IgE test alone are sufficient to confirm or refute a diagnosis of peanut allergy in a tertiary allergy clinic population of peanut-sensitised, peanut-naïve children with a history of egg allergy. However, Ara h 2-specific IgE testing does improve clinical utility when used in combination and can help reduce the number of children who require an oral provocation challenge.

Recommendations

Children with a skin prick test wheal diameter ≥6mm or a whole peanut-specific IgE concentration of ≥1.08 kUA/ L in conjunction with a positive Ara h 2-specific IgE concentration of ≥0.39 kUA/L could potentially be excluded from a peanut oral provocation challenge and be diagnosed as peanut allergic. Children with a skin prick test wheal diameter ˂6mm require further investigation, which can be optimised using Model 4 above. The classification of all children with both a peanut skin prick test wheal diameter of ˂6mm and an Ara h 2-specific IgE concentration of ˂0.39 kUA/L as peanut tolerant may significantly reduce the number of

children in this group of children who would require an oral provocation challenge, although this would place a small percentage of children at risk. The identification of other factors associated with a diagnosis of peanut allergy to help elucidate the peanut allergic status of this group of children, such as persistent egg allergy, are a potential focus for future research.

Chapter 6

Discussion and Conclusions

Jimmy Carter, US President 1977-81 was a peanut farmer. The presidential aeroplane, Airforce One, was dubbed 'Peanut One'.
https://commons.wikimedia.org/wiki/File:
Former_President_and_First_Lady_Carter_wave_from_their_aircraft.jpeg

CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 General discussion

This prospective study aimed to investigate the diagnostic value of measuring Ara h 2-specific IgE concentrations in predicting a clinical reaction to peanut in peanut-naïve children with a history of egg allergy. The performance of whole peanut- and Ara h 2-specific IgE concentrations and peanut skin prick test results in the diagnosis of peanut allergy were compared in a study of 101 children peanut-sensitised children with a history of egg allergy attending a tertiary paediatric allergy clinic. This is the first study to examine the performance of Ara h 2-specific IgE concentrations within this frequently encountered high-risk population. Analyses were conducted for the two primary groups, peanut allergic and peanut tolerant children. These groups were further divided into subgroups of peanut allergic children; those with challenge-proven allergy or tolerance, known peanut allergy, whole peanut test results above previously published 95% positive predictive values and resolved peanut allergy. The major finding of this study was that the measurement of Ara h 2-specific IgE concentrations performed better than whole peanut-specific IgE concentrations and skin prick testing in the diagnosis of peanut allergy in this study population of egg-allergic, peanut-sensitised children attending a tertiary paediatric allergy clinic. The performance was less helpful for the analysis of subgroups within the immunological grey area. However, Ara h 2-specific IgE concentration testing was best employed as part of a two-step approach diagnostic algorithm in conjunction with peanut skin prick testing.

The prevalence of peanut allergy in the Bristol egg-allergic study population was 31%. The association between egg allergy and peanut allergy is well recognised. In the LEAP study egg allergy was recognised to be the most important risk factor for the development of peanut allergy (Du Toit et al., 2008). Egg allergy is common in infants and young children and therefore the burden placed upon allergy services by this population is considerable (Savage et al., 2007). The three most common ways in which egg-allergic, peanut-sensitised children may present has been described as 1) following an adverse reaction associated with peanut consumption; 2) with peanut-sensitisation being an incidental finding on a mixed food allergen-specific IgE panel in primary care or 3) via referral for a clinic review to exclude peanut allergy in children with eczema or other food allergy prior to introduction (Lange et al., 2014). Recent changes in management aimed at the prevention of peanut allergy have prompted a need to clarify a child's peanut allergy status as early in life as possible (Du Toit et al., 2008, Du

Toit, 2015, Perkin et al., 2016). Currently, parents of children with negative tests to whole peanut are advised to cautiously introduce peanut into their child's diet and to ensure regular ingestion approximately three times per week to prevent the development of sensitisation and allergy. Considerable clinical acumen is necessary to addressing the diagnosis and management of possible peanut allergy in high-risk, egg allergic children. Children with whole peanut-specific IgE concentrations above the widely accepted 95% positive predictive value are advised that they are highly likely to be peanut allergic and are diagnosed as such and managed accordingly. Children over 12 months of age who are identified as peanut-sensitised and have skin prick test wheal diameters between 3mm and 7mm are counselled and offered an oral peanut provocation challenge. There is an urgent need to identify diagnostic tests that may assist in optimising the existing clinical service.

6.2 The NutCracker Study findings

In the present study, findings were in line with most other studies suggesting Ara h 2-specific IgE concentrations to be better than whole peanut-specific IgE concentrations and peanut skin prick testing at discriminating between peanut allergic and peanut tolerant children (Asarnoj et al., 2010a, Ebisawa et al., 2015, Ebisawa, 2012, van Erp et al., 2016, Codreanu et al., 2011, Pedrosa et al., 2012, Vereda et al., 2011). One UK study reported Ara h 2-specific IgE testing to have an area under the curve of 0.99 using the manufacturer's cut-off value of 0.35kUA/L (Nicolaou et al., 2011). Another study identified the threshold of 0.29kUA/L as having 93% sensitivity and 96% specificity (Codreanu et al., 2011). In contrast, some studies have reported peanut-specific IgE concentrations to be superior with a higher area under the curve (Kim, 2016).

Children assigned to the peanut allergy group were those with challenge-proven peanut allergy, a previous allergic reaction to peanut confirmed by skin prick or whole peanut-specific IgE testing, or a positive peanut skin prick test or whole-peanut specific IgE concentration above previously published positive predictive values. 56(80%) of allergic children had a positive Ara h 2-specific IgE concentration compared with only 6% of tolerant children. Allergic children had a median Ara h 2-specific IgE concentration of 4.8kUA/L compared with 0.34kUA/L for tolerant children. A higher median Ara h 2-specific IgE concentration compared with whole peanut-specific IgE has been previously reported (Kim, 2016). Median Ara h 2-specific concentrations in the current study were influenced by two subgroups; children with known peanut allergy (28.2kUA/L) and those with whole peanut test results above previously published positive predictive values (8.kUA/L) 28.2 kUA/L. Ara h2-testing had the highest

positive likelihood ratio of all three tests at 12.62 which translates to a child with clinically relevant peanut allergy being approximately 12 times more likely to have a positive Ara h-2 specific IgE concentration than an asymptomatic peanut-sensitised child. This compares favourably with the positive likelihood ratios for peanut skin prick testing and whole peanut-specific IgE concentrations, which were 1.25 and 1.32 respectively. A systematic review of 21 paediatric studies by Klemans et al also reported Ara h 2-specific IgE testing to have the highest positive likelihood ratio, and found equivalent negative likelihood ratios for all three tests as found in this study population (Klemans et al., 2015). The lowest subgroup positive likelihood ratio for Ara h 2-specific IgE concentrations was for children with challenge-proven peanut allergy, although at 5.81 this remained superior to those for whole peanut tests.

Some tolerant children did have positive Ara h 2-specific IgE concentrations, which has been the finding in previous studies. A study of UK peanut-sensitised schoolchildren identified 80 out of 81 children to have positive specific IgE concentrations to Ara h 2 (Nicolaou et al., 2011). A further study reported 26% of sensitised, tolerant subjects to have positive Ara h 2-specific IgE whilst another reported this figure to be 10% whilst another study has reported that no tolerant subject demonstrated positive specific IgE to any seed storage protein (Lopes de Oliveira, 2013, Ackerbauer et al., 2015, Astier et al., 2006). In the present study, a negative result increased the probability of a child being tolerant but could not be relied upon to predict peanut tolerance as several allergic children also had a negative result. Children with a negative Ara h 2-specific IgE concentration therefore require further investigation which may include the measurement of specific IgE to other peanut components but which in many cases remains likely to culminate in an oral provocation challenge. The clinical utility of measuring whole peanut-specific IgE concentrations was found to be limited. All peanut-tolerant children had Ara h 2-specific IgE concentrations of Grade 2 or below which may be helpful when reviewing a child in clinic.

In the present study, analysis of whole peanut-specific IgE concentrations identified that a concentration of Grade 4 or above is likely to indicate peanut allergy. This compares with previously published 95% positive predictive values, with the cut-off point for a Grade 4 serum-specific IgE classification being 15.49 kUA/L (Sampson and Ho, 1997, Kim, 2016). A previous UK study has also identified this cut-off value to be applicable to their study population, yielding 96.2% specificity (Nicolaou et al., 2011). Children with a whole peanut-specific IgE above 15kUA/L should be excluded from oral provocation challenge unless their individual sensitisation profile prompts further investigation. All peanut tolerant children, with

the exception of a single outlier, had a level of Grade 3 or below. 39(57%) of peanut allergic children also had concentrations of Grade 3 or below. This confirms the reduced diagnostic accuracy of whole peanut-specific IgE concentrations compared with Ara h 2-specific IgE concentrations in discriminating peanut allergy fro tolerance in peanut-naïve individuals with test values below the published positive predictive value. Additionally, many peanut tolerant children had a positive whole peanut-specific IgE level, which equates with the findings of other studies. Ackerbauer et al reported 75% of tolerant individual patients to have positive whole peanut-specific IgE concentrations (Ackerbauer et al., 2015). Allergic children had a median whole peanut-specific IgE concentration of 11.6 kUA/L compared with tolerant children who had a median whole peanut-specific IgE concentration of 0.63 kUA/L. As with Ara h 2-specific IgE testing, the results were affected by the inclusion of two subgroups of children; those who had known peanut allergy (47.8 kUA/L) and those with whole peanut test values above previously published positive predictive values (18 kUA/L). Children with challenge-proven peanut allergy or tolerance had comparable median whole peanut-specific IgE levels of 1.0 kUA/L and 1.2 kUA/L respectively confirming it to be an unhelpful test in the management of egg-allergic, peanut-naïve children sitting within the immunological grey area. Previously published studies concur that it has become unreasonable to preserve the measurement of whole-peanut specific IgE concentrations as an accurate test to discriminate allergy from tolerance (Martinet et al., 2016, Klemans, 2013, Wainstein et al., 2007, Aalberse et al., 2013). Unfortunately, the peanut skin prick test was similarly found to be a poor discriminator between allergic and tolerant children despite being helpful in the identification of peanut-sensitised children at risk of peanut allergy.

Skin prick testing remains the primary first line assessment tool for the review of egg-allergic children at risk of egg allergy. Despite its poor ability to predict peanut allergy in this population of children the negative predictive value using the cut-off value of <3mm was excellent at 100%. The association of a negative peanut skin prick test with tolerance concurs with earlier studies (POST, 2004). This test is therefore useful in eliminating a diagnosis of peanut allergy in egg allergic children, even if they have a positive whole peanut-specific IgE concentration. There was a significant difference between the two groups of peanut allergic and peanut tolerant children with allergic children having a mean wheal diameter of 10mm and tolerant children having a mean wheal diameter of 4mm although the results were influenced by the inclusion of the subgroups of children with peanut skin prick test or whole peanut-specific IgE concentrations above the 95% positive predictive values and children with known peanut allergy. The inclusion of the study subgroups is further examined in Section 6.4

below. Mean skin prick wheal diameters for these subgroups were 11mm and 7mm respectively. Subgroups of children with challenge-proven peanut allergy and peanut tolerant children both had mean peanut wheal diameters of 4mm. A positive peanut skin prick test was unable to discern between allergy and tolerance, being present in all 67 (100%) allergic children tested and 25 (83%) tolerant children. Children with a negative skin prick test to peanut below the manufacturer's cut-off value should introduce peanut cautiously at home rather than being subject to an oral provocation challenge whilst those with a positive peanut skin prick test therefore require further investigation. Likelihood ratios were constructed to assist with the prediction of oral provocation challenge outcome, which is of particular value for children within this immunological grey area.

Likelihood ratios for peanut skin prick testing and the measurement of whole-peanut and Ara h 2-specific IgE concentrations confirmed the pre-test probability of an egg-allergic child attending the tertiary paediatric allergy clinic at Bristol Royal Hospital for Children having peanut allergy to be 69%. A child in this group will have a higher relative risk for peanut allergy than a child without egg-allergy attending the clinic. Post-test probability was higher for all three tests. Ara h 2-specific IgE testing performed had the highest post-test probability of 97%, whilst whole peanut-specific IgE testing gave a post-test probability of 75% and skin prick testing gave a post-test probability of 74%. Ara h 2-specific IgE testing also gave the best post-test probability for the analysis of subgroups. Even for challenged children where pre-test probability was lowest at 21%, the post-test probability rose to 60%. The post-test probability for peanut skin prick testing and whole peanut-specific IgE concentrations were far less useful at 23% and 25% respectively. This confirms that for children within the immunological grey area who require an oral provocation challenge, Ara h-2 is the only test that is of any benefit in predicting possible outcome. However, its clinical utility does have limitations. For example, a post-test probability of 60% is too low for the clinician to be able to make a diagnosis on the basis of the Ara h 2-specific IgE concentration alone, although it does provide them with information regarding the chance of each child reacting on oral provocation challenge which they can relay to the child's parents or guardians when taking consent for the procedure.

There may be some clinical utility for Ara h-2 testing for the small number of children within the positive predictive value subgroup who have a negative Ara h 2-specific IgE concentration as this may indicate tolerance. This study identified a single peanut-tolerant child with a very highly positive whole peanut-specific IgE concentration of 51.6kUA/L and a negative Ara h 2-specific IgE concentration. The clinician who referred this infant chose to do so as he had a

small skin test response of 3mm and a negative Ara h 2-specific IgE, in the presence of a high total IgE level of 4556 kUA/L. The child had a clinical history of legume allergy and the clinician felt that his peanut-sensitisation might have been primarily related to cross-reactivity. Sensitisation to subunits of the 11S globulins present in legumes has been recognised in peanut-allergic patients (Nicolaou and Custovic, 2011).There was also a peanut-tolerant child with a high positive whole peanut-specific IgE concentration of 10.9 kUA/L and a negative Ara h 2-specific IgE concentration. Other studies have also identified this phenomena (Martinet et al., 2016). There were five further children from the positive predictive value subgroup who had a negative Ara h 2-specific IgE concentration who did not undergo an oral provocation challenge because their chance of tolerance was so low. Two of these children were excluded due to their whole peanut-specific IgE concentrations being 81.2 and 19.6 kUA/L whilst three others had been excluded on the basis of having skin prick tests of 8mm or above. These three children had whole peanut-specific IgE concentrations of 1.22, 6.7 and 14.3 kUA/L. In the light of the current study, it would now seem reasonable to re-evaluate these children with a view to offering them an oral provocation challenge providing they were aware of the possible low chance of them proving tolerant. In contrast, it is important to remember that four children with challenge proven peanut allergy had negative Ara h 2-specific IgE concentrations and low whole peanut-specific IgE concentrations. Some families may feel that the benefit of potentially removing the diagnosis of peanut allergy outweighs this risk. This re-evaluation could include specific IgE testing to other peanut components.

An alternative explanation for peanut-allergic children having a positive whole peanut-specific IgE concentration and a negative Ara h 2- specific IgE concentration may be that they are sensitised to a component other than Ara h 2 (Atkinson, 1992). Other studies have proposed further specific IgE testing to additional peanut components, especially for children with a history suggestive of birch pollen allergy (Martinet et al., 2016). Ara h 8 is a Bet v 1 homologue and has been shown to be the major allergen in children with combined birch pollen and peanut allergy. These children appear to be of a different phenotype with their reaction to peanut tending to be mild (Asarnoj et al., 2010b, Mittag et al., 2004). Ara h 9 is important for Mediterranean patients and in a study of Spanish patients was reported to be the immunodominant allergen in 60% of patients, with only 42% recognising Ara h 2 (Vereda et al., 2011). Its applicability to UK school children is as yet unknown. The cost of multiple peanut-component testing for all patients undergoing clinical review would however be a very expensive way of identifying peanut allergy in such a small sample of patients and should be reserved for discordant children. The health economic implications of new tests must always

be considered. The cost of testing for multiple peanut components and reviewing children at multiple outpatient appointments is likely to be little cheaper than an oral provocation challenge.

6.3 The biological mechanisms supporting Ara h 2-specific IgE concentration testing

Analysis of the study results has identified the measurement of Ara h 2-specific IgE concentrations to be the best test for the prediction of peanut allergy in peanut-sensitised, peanut-naïve children with a history of egg allergy. The component Ara h 2 is a robust structure of five α-helices arranged in a superhelix, connected by several loops and stabilised by four disulfide bridges. It is not easily denatured, which explains why it is an immunodominant allergen. In the US, more than 95% of individuals with peanut allergy have demonstrated positive Ara h 2-specific IgE concentrations indicating possible clinical utility (Palmer et al., 2005, Scurlock and Burks, 2004, Koppelman, 2004, Zhou et al., 2013). In comparison, whole peanut-specific IgE and skin prick testing are based on more crude extracts which leads to the recognition of specific IgE antibodies to any peanut component resulting in a positive test results. Some of these peanut components have a comparatively innocent clinical profile and react with other homologous proteins such as pollens. Studies have identified that individuals who only have detectable specific IgE to other components such as Ara h 8 or Ara h 9 are frequently asymptomatic, or prone to very mild symptoms. The reason why whole peanut-specific IgE tests and skin prick tests perform less well is that they are non-selective and detect the presence of specific IgE to all components. Whole peanut skin prick tests and specific IgE assays contain several IgE antibody-binding determinants. This includes those which are not specifically associated with peanut allergy such as Bet v 1 homologues and other pollen cross-reactive determinants (Codreanu et al., 2011). Cross-reactive carbohydrate determinants (CCDs) have been implicated as being responsible for peanut-sensitisation in peanut tolerant, grass-sensitised patients (Guilloux et al., 2009). This results in many tolerant individuals testing positive. This study has demonstrated that a proportion of peanut tolerant children have positive Ara h 2-specific IgE and reasons for this are not entirely clear. It may be that the testing is still not sufficiently developed and that further future developments in component-resolved diagnostic testing will result in improved accuracy. For example, ten epitopes of Ara h 2 have been mapped and it may be that some of these are associated with peanut allergy whilst others may be more commonly associated with asymptomatic peanut-sensitisation (Barre, 2005).

It has been proposed that children who exhibit binding to multiple peanut epitopes are more sensitive (Flinterman et al., 2008). The presence of binding to multiple peanut epitopes has also been associated with an increased severity in allergic reactions (Shreffler et al., 2004, Astier et al., 2006). In contrast, a study of UK schoolchildren identified that 39 peanut tolerant children demonstrated positive specific IgE concentrations to all peanut components (Nicolaou et al., 2011). One small study of 15 peanut allergic and 16 tolerant patients examined eight immunodominant sequential epitopes on the seed storage proteins Ara h 1,2 and 3. Most of the allergic individuals demonstrated specific IgE binding to three immunodominant epitopes on Ara h 2 whilst these were recognised by less than 10% of the tolerant individuals, regardless of their whole peanut-specific IgE concentrations (Astier et al., 2006). Ten of the 16 tolerant individuals had resolved peanut allergy suggesting that if resources allowed, then further future research into epitope mapping may be valuable in the prediction of peanut tolerance among children with confirmed peanut allergy. Examination of the clinical utility of Ara h 2-specific IgE concentrations in the diagnosis of peanut allergy in egg-allergic, peanut-sensitised children has raised a number of potential implications for current practice.

6.4 Study applicability

It is important that the findings of any study are considered in the light of the study population to which they apply. The sample of children in this study is representative of the local population; largely white British with approximately 16% of families deriving from an ethnic minority group. The ethnic minority children in this study were primarily of Polish, Somalian and Indian sub-continent heritage. The age of children in this sample is also representative of the spread of children attending the clinic. Egg allergy may take several years to resolve, evidenced by the large number of children in this study population demonstrating persistent egg allergy. The allergy clinic does review a large number of infants presenting with egg allergy and this population is slightly under-represented within this study population with only 14% of subjects being infants under two years of age. The age range of study children participating does reflect the surprisingly large number of children and adolescents who have actively avoided peanut. For some, especially the older teenagers, avoidance was on the basis of previous skin prick testing in early childhood, which had demonstrated peanut sensitisation subsequently interpreted as allergy without further investigation. In the light of the LEAP study research, it is now known that such unnecessary peanut avoidance will have resulted in the development of peanut allergy in some children (Du Toit, 2013). The problem of peanut avoidance among siblings of allergic children has been previously recognised as a problem (Lavine et al., 2015). Peanut avoidance was also often recommended by the referring GP or

due to parental anxiety. The LEAP study has highlighted the importance of early introduction of peanut among these children yet NHS paediatric allergy services are constantly under pressure and under resourced (RCP, 2003). Improved diagnostic testing is urgently required as a means of addressing the needs of the local population for the prevention of peanut allergy in future children. The need to evaluate the cut-off values for varying study populations, age groups and geographical reasons is widely accepted (van Erp et al., 2016). The primary analysis of the present study was for two groups of peanut allergic and tolerant children. These two groups comprise the entire clinic population. It would have been simpler to have only included children with challenge-proven allergy or tolerance in this study but this would not have been representative of clinical practice.

There is an urgent need to expedite the diagnostic process among high-risk children to reduce the burden of allergy for future generations. The clinic population comprises several different subgroups of egg-allergic children potentially at risk of peanut allergy. The present study included the subgroups of children with known peanut allergy and those with test values above the 95% positive predictive value to ensure that the study population comprised all the cohorts of egg-allergic peanut-sensitised children the clinician is likely to encounter. However, the results presented confirm that differences do exist between subgroups and whilst it is important that these are recognised when managing each individual child, this inclusion of subgroups does complicate the analysis of data.

The results for the subgroup of children with known peanut allergy were the most surprising. It had been anticipated that results would be comparable with those obtained from peanut-sensitised egg-allergic children who had never consumed peanut. Data comparison between subgroups identified both whole-peanut and Ara h 2-specific IgE concentrations to be significantly higher in children who had experienced a reaction in the community. These two groups of children are therefore not directly comparable and their inclusion has resulted in an unexpected limitation upon this study. However, children with known peanut allergy were a very small group of children.

The inclusion of three children with resolved peanut allergy could also be criticised. These children had been recruited into the study initially as part of the subgroup of children with known peanut allergy. Their very low whole peanut-specific IgE concentrations and skin prick test wheal diameters prompted the need for further investigation which resulted in the creation of this further subgroup.

The subgroup of children with whole peanut test results above previously published positive predictive values were not challenged. This could be criticised and would score poorly using the QUADAS-2 tool utilised above, but is in line with the majority of studies critiqued above in the Literature Review. As this is a study of usual care, this is also in line with current national practice. Furthermore, the large number of these children demonstrates the large proportion of egg-allergic children evaluated in a tertiary allergy clinic who will fall into this group. The majority of these children will not be challenged. This study proposes that there should be exceptions to this especially for children who have a negative Ara h 2- specific IgE concentration as reported in earlier studies (Ackerbauer et al., 2015). Lieberman et al examined 31 individuals with a whole peanut-specific IgE concentration of ≥15 kUA/L or above. All 28 with elevated Ara h 2-specific IgE concentrations failed an oral provocation challenge, whilst 3 with negative Ara h 2- specific IgE concentrations completed an oral provocation challenge without reaction despite having positive whole peanut-specific IgE concentrations of 2.1, 36.59 and 46.89 kUA/L (Lieberman, 2015). Children with discordant whole peanut- and Ara h 2-specific IgE concentrations warrant further evaluation, and may benefit from a more detailed assessment of their peanut-component profile but this remains an area in need of further research.

6.5 Implications of study findings and relevance to clinical practice

The current study has confirmed that there is no longer a role for the routine use of whole-peanut specific IgE concentrations in the diagnosis of peanut allergy in this population and that this should be replaced by the introduction of the routine measurement of Ara h 2-specific IgE concentrations instead. When used in isolation, Ara h 2-specific IgE concentrations were unable to replace the need for an oral peanut provocation challenge for the majority of egg-allergic, peanut-naïve children who had peanut-sensitisation test values below the widely accepted 95% positive predictive value. The measurement of Ara h 2-specific IgE concentrations however was a useful complementary tool for clinicians. There may be a role for testing for multiple peanut components but this should be used judiciously as it incurs additional costs and further research into the clinical utility of this approach is needed.

The measurement of Ara h 2-specific IgE concentrations as an additional tool for the diagnosis of peanut allergy within the Bristol Royal Hospital for Children paediatric allergy clinic has been advantageous. There are several children with high whole peanut-specific IgE or skin prick tests, albeit below the previously published positive predictive values, who few clinicians

would previously have been confident to challenge. A negative Ara h 2-specific IgE concentration within this group of children has led to a general change in behaviour, with an increase in the number of oral provocation challenges being offered. A reluctance to challenge children may inflict a peanut allergy diagnosis on a significant number of tolerant children. Clinicians working in smaller centres frequently perform fewer challenges than those working in tertiary centres, with many children being excluded from challenge unnecessarily. The growth of nurse-led allergy clinics may also in some centres be associated with a lack of confidence in challenging certain children due to a greater reliance upon diagnostic algorithms and guidelines rather than clinical acumen.

An improved approach to the diagnosis and management of these children would be to employ the models discussed earlier to reduce the number of oral provocation challenges necessary. The development of two stepwise approach models described above (Models 2 and 4) optimised the use of available tests. This is very useful in the tertiary paediatric allergy clinic where specific guidelines for the use of Ara h 2-specific IgE are not currently available and where children are reviewed by clinicians of all levels of experience, including specialist trainees and clinical nurse specialists. Clinicians may behave differently, partly dependent upon their level of experience. Both models require all egg-allergic, peanut-naïve children to undergo skin prick testing to identify peanut-sensitised children. The high negative predictive value for peanut skin prick testing highlights this test to be of great value. However, its value among a peanut-naïve study population primarily lies in the elimination of peanut allergy rather than in making a definitive diagnosis. The results of this study prompt the recommendation for the use of peanut skin prick testing as the initial screening test for use in clinic when reviewing egg-allergic, peanut-naïve infants and children. Children with a negative skin prick test to peanut below the manufacturer's cut-off value can cautiously introduce peanut at home. If skin prick testing cannot be performed for some reason, as is the case when a child has taken antihistamines for example, then an alternative approach will be needed.

Model 2, the two-step diagnostic algorithm which uses skin prick testing to peanut as the first step, is recommended for the management of egg-allergic, peanut-naïve children attending clinic who are identified as having a skin prick test wheal diameter of 6mm or above. These children then require venepuncture for Ara h 2-specific IgE testing. If a child's Ara h 2-specific IgE concentration is above the 0.39KAU/L cut-off value, the child can be given the diagnosis of peanut allergy with the knowledge that a small number of children may be misclassified. Future follow up of peanut allergic children every two years may eventually led to the

identification of tolerant children who have fallen into this group. Children who have an Ara h 2-specific IgE concentration below the 0.39kUA/L cut-off are more likely to be peanut tolerant than allergic but will still require an oral provocation challenge, to prevent those children misclassified as tolerant experiencing a potentially severe allergic reaction in the community.

Model 4 can be implemented for the management of children with a positive skin prick test which is less than 6mm. Children with an Ara h 2-specific IgE concentration of ≤0.39 kUA/L would be classified as peanut tolerant and those with a concentration above this level would require an oral provocation challenge. This model is anticipated to reduce the number of children who will require an oral provocation challenge although it would place a small number of children at risk. This risk could be reduced by the use of supervised feed clinics, where children attend an outpatient appointment where they ingest a standard portion of peanut under minimal supervision in the safety of a hospital environment. The use of the model in routine clinical practice could be simplified by the introduction of an app or computerised algorithm. Now that NHS hospitals are moving towards paperless systems, iPads are being routinely introduced into many clinics which could easily facilitate this introduction.

A negative Ara h 2-specific IgE concentration is not always associated with tolerance and children with discordant results will require further investigation. The reviewing clinician should consider additional testing to other components should be considered when Ara h 2 is negative in peanut-naïve children with birch pollen sensitivity. There may be a role for prick testing to birch as a useful first line test in the evaluation of children with positive whole-peanut testing and negative Ara h 2-specific IgE as this may possibly reduce the number of blood tests to which the child is subjected, whilst also reducing costs.

6.6 Study considerations

The contemplation of a change in practice based on the findings of a research study will always have implications. These considerations include factors such as logistical or health economic considerations. One consideration in this study is the cost of additional component testing. The cost of Ara h 2-specific IgE concentration testing in addition to routine costing is considerable when applied to a large tertiary allergy clinic. The findings of the present study recommend replacing whole-peanut specific IgE testing with Ara h 2-specific IgE concentrations. The use of further component testing is recommended only on a case-by-case basis. This study restricted component-testing to Ara h 2 largely due to cost but also following

review of the literature which does not pose a very strong argument for multiple peanut component testing in all children. This was a study of routine clinical care and such indiscriminate testing is unlikely to ever be an NHS recommendation. Additionally, cross-reactivity rates have been demonstrated to be low among very young children. As this study had initially planned to include a larger number of infants, evaluation of Ara h 9 was anticipated to be of limited interest (Dang et al., 2012). Bristol has a very low Mediterranean population, suggesting that Ara h 9 is unlikely to be an immunodominant allergen (Asarnoj et al., 2012b, Mittag et al., 2004). It is unlikely therefore that this decision had any detrimental effect upon the study outcome.

A second minor consideration that may have restricted the ability of the study to make recommendations specifically for the management of infants under the age of two years was the small number of infants included. However, the age of included children does reflect the referral process within the Southwest region. The waiting time following receipt of a GP referral for a new patient appointment exceeds the 18 week pathway and any child referred on for an oral provocation challenge is likely to experience a further six month wait prior to the procedure. For this reason, the opportunity to challenge children under the age of two is limited.

A further consideration is the varying quality of skin prick test extracts and specific IgE assays. The present study used high quality extracts but it should be recognised that the recommended stepwise models may perform differently with different skin test reagents of *in vitro* specific IgE testing systems. Further validation of the model may be required in other study centres.

6.7 Study strengths and limitations

There were some limitations to the current study, most of which relate to this being a study of routine clinical care, which is a consideration for clinical practice and the delivery of any recommendations. There was a time delay of several weeks between venepuncture and the oral provocation challenge. Some previous studies were able to take blood immediately prior to oral provocation challenge but if a second blood sample had been taken in the present study, this process would not replicate standard care, and therefore the findings would be less applicable to current practice. However, it is unlikely that there would be a dramatic difference in specific IgE concentrations between their clinical appointment and their oral provocation challenge.

The most important limitation to this study was the decision not to challenge children with whole peanut-specific IgE concentrations or skin prick test wheal diameters above previously published 95% positive predictive values, which was an ethical restriction. The exclusion of this cohort of children is not unusual among study populations with these children frequently being labelled as peanut allergic for safety purposes (Kim, 2016). The number of children in this subgroup who may have been misclassified as allergic rather than tolerant will be small. Within the HealthNuts study all peanut-sensitised children were challenged regardless of their whole peanut-specific IgE or skin prick test wheal measurements, and if children with test results above the positive predictive value had been excluded then the misclassification rate among children would have been 3% (Dang et al., 2012). The current study examined optimal ways of utilising available testing within clinical practice, working within commonly encountered restrictions. It would not be logistically possible, or safe, within routine clinical practice to challenge all peanut-naïve children falling within this positive predictive value subgroup. The study has identified optimal ways of managing this subgroup of children as previously discussed.

A further limitation was that all provocation challenges were open challenges, as opposed to double-blind placebo-controlled food challenges. This was again reflective of routine clinical practice but the robust scoring system ensured that the oral provocation challenges were performed in an objective manner.

One strength of this study is that it was a prospective study, which compares favourably with several other retrospective studies described in chapter 2. It is also the first study to specifically evaluate the diagnostic utility of Ara h 2-specific IgE concentrations in a clinic population of egg-allergic, peanut-sensitised children who have never knowingly ingested peanut. This is of prime importance given the recent publication of the LEAP trial, which examined the effectiveness of the randomised consumption of peanut in preventing allergy among high-risk egg allergic children, and in children with eczema (Du Toit, 2015). Allergy services are consequently under pressure to facilitate the early introduction of peanut to high-risk infants but unfortunately have inadequate resources for the assessment of these children necessary to enable the safe introduction of peanut. Therefore tests able to better discriminate between peanut allergy and tolerance would be of considerable value.

6.8 Implications for future research

This study has highlighted several areas for the focus of future research. Further research to identify other factors associated with peanut allergy which would complement use of the diagnostic model are required to elucidate the peanut allergy status of peanut-sensitised, peanut-naïve children with a history of egg allergy. This should include investigation of factors such as persistent egg allergy, age and pollen-sensitisation profiles. A valuable and interesting focus for future research includes the examination of the possible potential role of skin testing to pollens in the identification of peanut tolerant children.

Evaluation of the small number of children with whole peanut-specific IgE or skin prick test values above the positive predictive value also requires clarification. Previously published studies do not concur on the clinical utility of multiple components or epitope mapping in the management of peanut-sensitised, peanut-naïve children. Further research will establish whether these children may benefit from further clarification of their sensitisation profile via the use of other peanut components or epitope mapping. Epitope mapping may also be of additional benefit for the identification of children who may have resolved peanut allergy. Further examination of the clinical utility of Ara h 2 epitope mapping for the prediction of peanut allergy resolution would have both patient and health economic benefits.

A further minor focus for future research would be to assess the efficacy and user-acceptability of the introduction of electronic diagnostic algorithms in the management of peanut-sensitised, peanut-naïve children. This could be examined in terms of cost-savings and compared with the existing management of this group of children to assess whether introduction of the model is associated with cost savings and a more streamlined and effective service. Finally, research into the applicability of the model to other high-risk populations of peanut-sensitised, peanut-naïve children is important. Other populations identified as being at high-risk of peanut-allergy include children with eczema and other populations where knowledge of a child's peanut allergy status are also important include siblings of peanut allergic children, and children with tree nut allergy who are peanut-sensitised and peanut-naïve.

The inclusion of the several subgroups of children within the current study has highlighted that results can vary considerably between these groups. It has been valuable to be able to examine these subgroups and identify where some of these differences lie. For clarity, and given the very small sample size of children within the known peanut allergy and resolved

peanut allergy subgroups, these two subgroups will be excluded from the journal paper to be submitted for publication. As the Allergy Department at Bristol Royal Hospital for Children has continued to record Ara h 2-specific IgE concentrations and peanut oral provocation challenge outcomes, this additional data from clinical practice will be added to the analysis prior to submission of the final paper for publication in place of the data obtained from analysis of the subgroups.

6.9 Conclusions

The results of this study clearly demonstrate the greater ability of sensitisation to Ara h 2 in distinguishing between peanut allergy and asymptomatic peanut sensitisation in egg-allergic, peanut-naïve children compared with diagnosis being based on skin prick testing or whole peanut-specific IgE concentrations. Analysis of subgroups identified a decrease in its clinical utility although it remained the best test. Unfortunately it performed least well for those children falling in the immunological grey area whose diagnosis was confirmed by a peanut oral provocation challenge. This is the group of children in whom an improved diagnostic test is most needed. The completion of this study has led to several recommendations.

Children with a negative skin prick test to peanut below the manufacturer's cut-off value are unlikely to have peanut allergy and do not require a peanut oral provocation challenge. Optimal cut-off values have been identified for the management and diagnosis of peanut allergy in high-risk children. This study suggests that whole peanut-specific IgE concentrations should not be used in isolation, as concentrations below the 15kUA/L have limited clinical utility and should be replaced by the measurement of Ara h 2-specific IgE concentrations. There was little clinical utility for the use of whole peanut-specific IgE concentrations except as a potential screening tool for peanut-sensitisation. Children with a whole peanut-specific IgE above 15kUA/L should be excluded from oral provocation challenge unless their individual sensitisation profile prompts further investigation. Whole peanut-specific IgE concentrations need not be measured for this purpose as the 95% positive predictive value of 8mm for skin testing can be used for this purpose. Optimal cut-off values were 6mm for skin prick test wheal diameters and 0.39kUA/L for the measurement of Ara h 2-specific IgE concentrations. These have greatest clinical utility when used as part of a two-step approach model which measures skin prick test wheal diameters to peanut followed by Ara h 2-specific IgE concentrations.

When used in isolation, although the predictive ability of Ara h 2-specific IgE concentrations is superior to those of existing tests for whole peanut, their use was unable to replace the need

for an oral peanut provocation challenge for the majority of egg-allergic, peanut-naïve children who had peanut-sensitisation test values below the widely accepted 95% positive predictive value. Paediatric allergy has not yet reached the stage where laboratory-based testing can replace the gold standard of the peanut oral provocation challenge.

References

References

Laerdal SimBaby.

AALBERSE, J. A., MEIJER, Y., DERKSEN, N., VAN DER PALEN-MERKUS, T., KNOL, E. & AALBERSE, R. C. 2013. Moving from peanut extract to peanut components: towards validation of component-resolved IgE tests. *Allergy,* 68**,** 748-56.

ACKERBAUER, D., BUBLIN, M., RADAUER, C., VARGA, E. M., HAFNER, C., EBNER, C., SZEPFALUSI, Z., FROSCHL, R., HOFFMANN-SOMMERGRUBER, K., EIWEGGER, T. & BREITENEDER, H. 2015. Component-resolved IgE profiles in Austrian patients with a convincing history of peanut allergy. *Int Arch Allergy Immunol,* 166**,** 13-24.

AGABRIEL, C., GHAZOUANI, O., BIRNBAUM, J., LIABEUF, V., PORRI, F., GOUITAA, M., CLEACH, I., GROB, JJ., BONGRAND, P., SARLES, J., VITTE, J. 2014. Ara h 2 and Ara h 6 sensitization predicts peanut allergy in Mediterranean pediatric patients. *Pediatr Allergy Immunol,* 25**,** 662-7.

AKOBENG, A. K. 2007. Understanding diagnostic tests 3: Receiver operating characteristic curves. *Acta Paediatr,* 96**,** 644-7.

APC, T. A. P. C. 2014. *Export peanut market* [Online]. Available: https://http://www.peanutsusa.com/about-peanuts/the-peanut-industry3/19-export-peanut-market.html [Accessed 01/06/2016 2016].

ASARNOJ, A., MOVERARE, R., OSTBLOM, E., POORAFSHAR, M., LILJA, G., HEDLIN, G., VAN HAGE, M., AHLSTEDT, S. & WICKMAN, M. 2010a. IgE to peanut allergen components: relation to peanut symptoms and pollen sensitization in 8-year-olds. *Allergy,* 65**,** 1189-95.

ASARNOJ, A., NILSSON, C., LIDHOLM, J., GLAUMANN, S., OSTBLOM, E., HEDLIN, G., VAN HAGE, M., LILJA, G. & WICKMAN, M. 2012a. Peanut component Ara h 8 sensitization and tolerance to peanut. *Journal of Allergy and Clinical Immunology,* 130.

ASARNOJ, A., NILSSON, C., LIDHOLM, J., GLAUMANN, S., STBLOM, E., HEDLIN, G., VAN HAGE, M., LILJA, G. & WICKMAN, M. 2012b. Peanut component Ara h 8 sensitization and tolerance to peanut. *J Allergy Clin Immunol,* 130**,** 468-72.

ASARNOJ, A., OSTBLOM, E., AHLSTEDT, S., HEDLIN, G., LILJA, G., VAN HAGE, M. & WICKMAN, M. 2010b. Reported symptoms to peanut between 4 and 8 years among children sensitized to peanut and birch pollen - results from the BAMSE birth cohort. *Allergy,* 65**,** 213-9.

ASERO, R., MISTRELLO, G. , RONCAROLO, D, ET AL 2002. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy,* 57.

ASTIER, C., MORISSET, M., ROITEL, O., CODREANU, F., JACQUENET, S., FRANCK, P., OGIER, V., PETIT, N., PROUST, B., MONERET-VAUTRIN, D.-A., BURKS, A. W., BIHAIN, B., SAMPSON, H. A. & KANNY, G. 2006. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. *The Journal of allergy and clinical immunology,* 118**,** 250-6.

ASTWOOD, J. D., LEACH, J.N., FUCHS, R.L. 1996. Stability of food allergens to digestion in vitro. *Natural Biotechnology,* 14**,** 1269.

ATKINSON, W. L., ORENSTEIN, W.A., KRUGMAN, S. 1992. The resurgency of measles in the United States, 1989-1990. *Ann Rev Med,* 43**,** 451-63.

BALLMER-WEBER, B. K., LIDHOLM, J., FERNANDEZ-RIVAS, M., SENEVIRATNE, S., HANSCHMANN, K.M., VOGEL, L., BURES, P., FRITSCHE, P., SUMMERS, C., KNULST, A.C., LE, T.,M., REIG, I., PAPADOPOULOS, N.G., SINANIOTIS, A., BELOHLAVKOVA, S., POPOV, T., KRALIMARKOVA, T., DE BLAY, F., PUROHIT, A., CLAUSEN, M., KOWALSKI, M.ML., ASERO, R., DUBAKIENE, R., BARREALES, L., MILLS, C., VAN REE, R., VIETHS, S. 2015. IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall study. *Allergy*.

BARRE, A., BORGES, J., CULERRIER, R., ROUGE, P. 2005. Homology modelling of the major peanut allergen Ara h 2 and surface mapping of IgE-binding epitopes. *Immunology Letters,* 100**,** 153-158.
BARRE, A., SORDET, C., CULERRIER, R., RANCE, F., DIDIER, A. & ROUGE, P. 2008. Vicilin allergens of peanut and tree nuts (walnut, hazelnut and cashew nut) share structurally related IgE-binding epitopes. *Molecular immunology,* 45**,** 1231-40.
BECKER, W. M. & JAPPE, U. 2014. Peanut allergens. *Chem Immunol Allergy,* 100**,** 256-67.
BENHAMOU, A. H., CAUBET, J. C., EIGENMANN, P. A., NOWAK-WEGRZYN, A., MARCOS, C. P., RECHE, M. & URISU, A. 2009. State of the art and new horizons in the diagnosis and management of egg allergy. *Allergy*.
BERNARD, H., GUILLON, B., DRUMARE, M. F., PATY, E., DRESKIN, S. C., WAL, J. M., ADEL-PATIENT, K. & HAZEBROUCK, S. 2015. Allergenicity of peanut component Ara h 2: Contribution of conformational versus linear hydroxyproline-containing epitopes. *J Allergy Clin Immunol,* 135**,** 1267-74 e1-8.
BEYER, K., GRABENHENRICH, L., HARTI, M., BEDER, A., KALB, B., ZIEGERT, M., FINGER, A., HARANDI, N., SCHLAGS, R., GAPPA, M., PUZZO, L., ROBLITZ, H., MILLNER-UHLEMANN, M., BUSING, S., OTT, H., LANGE, L., NIGGEMANN, B. 2015. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. *Allergy,* 70**,** 90-98.
BLOM, W. M., VLIEG-BOERSTRA, B. J., KRUIZINGA, A. G., VAN DER HEIDE, S., HOUBEN, G. F. & DUBOIS, A. E. 2013. Threshold dose distributions for 5 major allergenic foods in children. *J Allergy Clin Immunol,* 131**,** 172-9.
BLUMCHEN, K., ULBRICHT, H., STADEN, U., DOBBERSTEIN, K., BESCHORNER, J., DE OLIVEIRA, L. C., SHREFFLER, W. G., SAMPSON, H. A., NIGGEMANN, B., WAHN, U. & BEYER, K. 2010. Oral peanut immunotherapy in children with peanut anaphylaxis. *J Allergy Clin Immunol,* 126**,** 83-91 e1.
BOCK, S. A., BUCKLEY, J., HOLST, A. & MAY, C. D. 1977. Proper use of skin tests with food extracts in diagnosis of hypersensitivity to food in children. *Clin Allergy,* 7**,** 375-83.
BOCK, S. A., SAMPSON, H. A., ATKINS, F. M., ZEIGER, R. S., LEHRER, S., SACHS, M., BUSH, R. K. & METCALFE, D. D. 1988. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol,* 82**,** 986-97.
BOUSQUET, J., LOCKEY, R. & MALLING, H. J. 1998. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol,* 102**,** 558-62.
BOYANO-MARTINEZ, T., GARCIA-ARA, C., DIAZ-PENA, J. M. & MARTIN-ESTEBAN, M. 2002. Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy. *J Allergy Clin Immunol,* 110**,** 304-9.
BREITENEDER, H. & MILLS, C. E. N. 2005. Plant food allergens--structural and functional aspects of allergenicity. *Biotechnol Adv,* 23**,** 395-9.
BROUGH, H. A., SIMPSON, A., MAKINSON, K., HANKINSON, J., BROWN, S., DOUIRI, A., BELGRAVE, D. C., PENAGOS, M., STEPHENS, A. C., MCLEAN, W. H., TURCANU, V., NICOLAOU, N., CUSTOVIC, A. & LACK, G. 2014. Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. *J Allergy Clin Immunol,* 134**,** 867-875 e1.
BROUGH, H. A., TURNER, P. J., WRIGHT, T., FOX, A. T., TAYLOR, S. L., WARNER, J. O. & LACK, G. 2015. Dietary management of peanut and tree nut allergy: what exactly should patients avoid? *Clin Exp Allergy,* 45**,** 859-71.
BROWN, S. J., ASAI, Y., CORDELL, H. J., CAMPBELL, L. E., ZHAO, Y., LIAO, H., NORTHSTONE, K., HENDERSON, J., ALIZADEHFAR, R., BEN-SHOSHAN, M., MORGAN, K., ROBERTS, G., MASTHOFF, L. J., PASMANS, S. G., VAN DEN AKKER, P. C., WIJMENGA, C., HOURIHANE, J. O., PALMER, C. N.,

LACK, G., CLARKE, A., HULL, P. R., IRVINE, A. D. & MCLEAN, W. H. 2011. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol,* 127**,** 661-7.
BURBANK, A. J. & BURKS, W. 2015. Food specific oral immunotherapy: a potential treatment for food allergy. *Expert Rev Gastroenterol Hepatol,* 9**,** 1147-59.
BURKS, A. W., JAMES, J.M., HIEGEL, A., WILSON, G., WHEELER, J.G., JONES, S.M., ZUERLEIN, N. 1998. Atopic dermatitis and food hypersensitivity reactions. *J Pediatr,* 132**,** 132-6.
BURKS, A. W., SHIN, D., COCKRELL, G., STANLEY, J.S., HELM, R.M., BANNON, G.A. 1997. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur J Biochem,* 245**,** 334-9.
CHASSAIGNE, H., NORGAARD, J.V., HENGEL, A.J. 2007. Proteomics-based approach to detect and identify major allergens in processed peanuts by capillary LC-Q-TOF (MS/MS). *J Agric Food Chem,* 55**,** 4461-4473.
CHEN, H., GAO, J. 2012. *Food allergen epitopes,* Nanchang, China.
CHEN, X., NEGI, S. S., LIAO, S., GAO, V., BRAUN, W. & DRESKIN, S. C. 2016. Conformational IgE epitopes of peanut allergens Ara h 2 and Ara h 6. *Clin Exp Allergy,* 46**,** 1120-8.
CHEN, X., WANG, Q., EL-MEZAYEN, R., ZHUANG, Y. & DRESKIN, S. C. 2013. Ara h 2 and Ara h 6 have similar allergenic activity and are substantially redundant. *Int Arch Allergy Immunol,* 160**,** 251-8.
CLARK, A. T., ISLAM, S., KING, Y., DEIGHTON, J., ANAGNOSTOU, K. & EWAN, P. W. 2009. Successful oral tolerance induction in severe peanut allergy. *Allergy,* 64**,** 1218-20.
CODREANU, F., COLLIGNON, O., ROITEL, O., THOUVENOT, B., SAUVAGE, C., VILAIN, A. C., COUSIN, M. O., DECOSTER, A., RENAUDIN, J. M., ASTIER, C., MONNEZ, J. M., VALLOIS, P., MORISSET, M., MONERET-VAUTRIN, D. A., BRULLIARD, M., OGIER, V., CASTELAIN, M. C., KANNY, G., BIHAIN, B. E. & JACQUENET, S. 2011. A novel immunoassay using recombinant allergens simplifies peanut allergy diagnosis. *International archives of allergy and immunology,* 154**,** 216-26.
CUMMINGS, A. J., KNIBB, R. C., ERLEWYN-LAJEUNESSE, M., KING, R. M., ROBERTS, G. & LUCAS, J. S. Management of nut allergy influences quality of life and anxiety in children and their mothers. *Pediatr Allergy Immunol*.
CUMMINGS, A. J., KNIBB, R. C., ERLEWYN-LAJEUNESSE, M., KING, R. M., ROBERTS, G. & LUCAS, J. S. A. 2010. Management of nut allergy influences quality of life and anxiety in children and their mothers. *Pediatric Allergy and Immunology,* 21.
DANG, T. D., TANG, M., CHOO, S., LICCIARDI, P. V., KOPLIN, J. J., MARTIN, P. E., TAN, T., GURRIN, L. C., PONSONBY, A.-L., TEY, D., ROBINSON, M., DHARMAGE, S. C., ALLEN, K. J. & HEALTHNUTS, S. 2012. Increasing the accuracy of peanut allergy diagnosis by using Ara h 2. *The Journal of allergy and clinical immunology,* 129**,** 1056-63.
DEAN, T., VENTER, C., PEREIRA, B., ARSHAD, S. H., GRUNDY, J., CLAYTON, C. B. & HIGGINS, B. 2007. Patterns of sensitization to food and aeroallergens in the first 3 years of life. *J Allergy Clin Immunol,* 120**,** 1166-71.
DESCOTES, J. & CHOQUET-KASTYLEVSKY, G. 2001. Gell and Coombs's classification: is it still valid? *Toxicology,* 158**,** 43-9.
DESHPANDE, S. S., NIELSEN, J. 1987. In vitro digestibility of dry bean (Phaseolus vulgaris L.) proteins: the role of heat stable protease inhibitors. *Journal of Food Science,* 52.
DREBORG, S. 1993. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy,* 48**,** 473-5.
DREBORG, S. 2001. Diagnosis of food allergy: tests in vivo and in vitro. *Pediatr Allergy Immunol,* 12 Suppl 14**,** 24-30.

DU TOIT, G. 2015. Randomized trial of peanut consumption in infants at risk for peanut allergy. *The New England Journal of Medicine* 372**,** 803-813.
DU TOIT, G., KATZ, Y., SASIENI, P., MESHER, D., MALEKI, S. J., FISHER, H. R., FOX, A. T., TURCANU, V., AMIR, T., ZADIK-MNUHIN, G., COHEN, A., LIVNE, I. & LACK, G. 2008. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol,* 122**,** 984-91.
DU TOIT, G. R., G. SAYRE, PH. PLAUT, M. BAHNSON, HT. MITCHELL, H. RADULOVIC, S. CHAN, S. FOX, A. TURCANU, V. LACK, G. 2013. Identifying infants at high risk of peanut allergy: the Learning Early About Peanut Allergy (LEAP) Screening study. *J Allergy Clin Immunol,* 131**,** 135-43.
DUNNGALVIN, A., DE BLOKFLOKSTRA, B. M., BURKS, A. W., DUBOIS, A. E. & HOURIHANE, J. O. 2008. Food allergy QoL questionnaire for children aged 0-12 years: content, construct, and cross-cultural validity. *Clin Exp Allergy,* 38**,** 977-86.
EBISAWA, M., MOVERARE, R., SATO, S., BORRES, M. P. & ITO, K. 2015. The predictive relationship between peanut- and Ara h 2-specific serum IgE concentrations and peanut allergy. *J Allergy Clin Immunol Pract,* 3**,** 131-2 e1.
EBISAWA, M., MOVERARE, R., SATO, S., MARUYAMA, N., BORRES, M.P. 2012. Measurement of Ara h 1-, 2-, and 3-specific IgE antibodies is useful in diagnosis of peanut allergy in Japanese children. *Pediatr Allergy Immunol,* 23**,** 573-581.
EGGESBO, M., BOTTEN, G. & STIGUM, H. 2001. Restricted diets in children with reactions to milk and egg perceived by their parents. *J Pediatr,* 139**,** 583-7.
EGGESBO, M., HALVORSEN, R., TAMBS, K. & BOTTEN, G. 1999. Prevalence of parentally perceived adverse reactions to food in young children. *Pediatr Allergy Immunol,* 10**,** 122-32.
EIGENMANN, P. A. & SAMPSON, H. A. 1998. Interpreting skin prick tests in the evaluation of food allergy in children. *Pediatr Allergy Immunol,* 9**,** 186-91.
EIWEGGER, T., RIGBY, N., MONDOULET, L., BERNARD, H., KRAUTH, M. T., BOEHM, A., DEHLINK, E., VALENT, P., WAL, J. M., MILLS, E. N. & SZEPFALUSI, Z. 2006. Gastro-duodenal digestion products of the major peanut allergen Ara h 1 retain an allergenic potential. *Clin Exp Allergy,* 36**,** 1281-8.
ELLER, E. & BINDSLEV-JENSEN, C. 2013. Clinical value of component-resolved diagnostics in peanut-allergic patients. *Allergy,* 68**,** 190-4.
EMMETT, S. E., ANGUS, F. J., FRY, J. S. & LEE, P. N. 1999. Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members. *Allergy,* 54**,** 380-5.
FALLON, P. G., SASAKI, T., SANDILANDS, A, ET AL 2009. A homozygous frameshift mutation in the mouse Fig gene faciliates enhanced percutaneous allergen priming. *Nat Genet,* 41.
FERNANDES, H., MICHALSKA, K., SIKORSKI, M. & JASKOLSKI, M. 2013. Structural and functional aspects of PR-10 proteins. *FEBS J,* 280**,** 1169-99.
FLINTERMAN, A. E., KNOL, E. F., LENCER, D. A., BARDINA, L., DEN HARTOG JAGER, C. F., LIN, J., PASMANS, S. G., BRUIJNZEEL-KOOMEN, C. A., SAMPSON, H. A., VAN HOFFEN, E. & SHREFFLER, W. G. 2008. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. *J Allergy Clin Immunol,* 121**,** 737-743 e10.
FLINTERMAN, A. E., VAN HOFFEN, E., DEN HARTOG JAGER, C. F., KOPPELMAN, S., PASMANS, S. G., HOEKSTRA, M. O., BRUIJNZEEL-KOOMEN, C. A., KNULST, A. C. & KNOL, E. F. 2007. Children with peanut allergy recognize predominantly Ara h2 and Ara h6, which remains stable over time. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology,* 37**,** 1221-8.
FLOKSTRA-DE BLOK, B. M., DUNNGALVIN, A., VLIEG-BOERSTRA, B. J., OUDE ELBERINK, J. N., DUIVERMAN, E. J., HOURIHANE, J. O. & DUBOIS, A. E. 2008. Development and validation of the self-administered Food Allergy Quality

of Life Questionnaire for adolescents. *J Allergy Clin Immunol,* 122**,** 139-44, 144 e1-2.
FLOKSTRA-DE BLOK, B. M., DUNNGALVIN, A., VLIEG-BOERSTRA, B. J., OUDE ELBERINK, J. N., DUIVERMAN, E. J., HOURIHANE, J. O. & DUBOIS, A. E. 2009. Development and validation of a self-administered Food Allergy Quality of Life Questionnaire for children. *Clin Exp Allergy,* 39**,** 127-37.
GARCIA-ARA, M. C., BOYANO-MARTINEZ, M. T., DIAZ-PENA, J. M., MARTIN-MUNOZ, M. F. & MARTIN-ESTEBAN, M. 2004. Cow's milk-specific immunoglobulin E levels as predictors of clinical reactivity in the follow-up of the cow's milk allergy infants. *Clin Exp Allergy,* 34**,** 866-70.
GELL, P. G. H., COOMBS, R.R.A. 1963. The classification of allergic reactions underlying disease. *In:* COOMBS, R. R. A., GELL, P.G.H. (ed.) *Clinical Aspects of Immunology.* Oxford: Blackwell Science.
GLAUMANN, S., NOPP, A., JOHANSSON, S. G., BORRES, M. P., LILJA, G. & NILSSON, C. 2013. Anaphylaxis to peanuts in a 16-year-old girl with birch pollen allergy and with monosensitization to Ara h 8. *J Allergy Clin Immunol Pract,* 1**,** 698-9.
GOLDMAN, M. 1998. Peanut allergy: how much peanut is too much? *In:* AMERICA, A. A. F. O. (ed.) *Chapter newsletter.* Maryland.
GRABENHENRICH, L., LANGE, L., HARTL, M., KALB, B., ZIEGERT, M., FINGER, A., HARANDI, N., SCHLAGS, R., GAPPA, M., PUZZO, L., STEPHAN, V., HEIGELE, T., BUSING, S., OTT, H., NIGGEMANN, B. & BEYER, K. 2016. The component-specific to total IgE ratios do not improve peanut and hazelnut allergy diagnoses. *J Allergy Clin Immunol,* 137**,** 1751-1760 e8.
GRUNDY, J., MATTHEWS, S., BATEMAN, B., DEAN, T. & ARSHAD, S. H. 2002. Rising prevalence of allergy to peanut in children: Data from 2 sequential cohorts. *J Allergy Clin Immunol,* 110**,** 784-9.
GUILLOUX, L., MORISSET, M., CODREANU, F., PARISOT, L. & MONERET-VAUTRIN, D. A. 2009. Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: roles of peanut and cross-reactive carbohydrate determinants specific IgE. *Int Arch Allergy Immunol,* 149**,** 91-7.
HALES, B. J., BOSCO, A., MILLS, K.L., HAZELL, L.A., LOH, R., HOLT, P.G., THOMAS, W.R. 2004. Isoforms of the major peanut allergen Ara h 2: IgE binding in children with peanut allergy. *Int Arch Allergy Immunol,* 135**,** 101-107.
HANSEN, C. S., DUFVA, M., BOGH, K. L., SULLIVAN, E., PATEL, J., EIWEGGER, T., SZEPFALUSI, Z., NIELSEN, M. & CHRISTIANSEN, A. 2016. Linear epitope mapping of peanut allergens demonstrates individualized and persistent antibody-binding patterns. *J Allergy Clin Immunol*.
HE, H., LIU, D., ZHANG, N., ZHENG, W., HAN, Q., JI, B., GE, F., CHEN, C. 2014. The PR10 gene family is highly expressed in *Iilium regale* Wilson during *Fusarium oxysporum* f. sp. *lilii* infection. *Genes Genom,* 36.
HELM, R. M., ERMEL, R.W., FRICK, O.L. 2003. Nonmurine animal models of food allergy. *Environmental Health Perspectives,* 111.
HERBERT, L. J. & DAHLQUIST, L. M. 2008. Perceived history of anaphylaxis and parental overprotection, autonomy, anxiety, and depression in food allergic young adults. *J Clin Psychol Med Settings,* 15**,** 261-9.
HILL, D. J., HEINE, R. G. & HOSKING, C. S. 2004. The diagnostic value of skin prick testing in children with food allergy. *Pediatr Allergy Immunol,* 15**,** 435-41.
HILL, D. J. & HOSKING, C. S. 2004. Food allergy and atopic dermatitis in infancy: an epidemiologic study. *Pediatr Allergy Immunol,* 15**,** 421-7.
HILL, D. J., HOSKING, C. S. & REYES-BENITO, L. V. 2001. Reducing the need for food allergen challenges in young children: a comparison of in vitro with in vivo tests. *Clin Exp Allergy,* 31**,** 1031-5.
HO, M. H., WOMG, W.H., HEINE, R.G., HOSKING, C.S., HILL, D.J., ALLEN, K.J. 2008. Early clinical predictors of remission of peanut allergy in children. *J Allergy Clin Immunol,* 121**,** 731-6.

HOURIHANE, J. O., RHODES, H.L., JONES, A.M., VEYS, P., CONNETT, G.J, 2005. Resolution of peanut allergy: a case-control study. *BMJ,* 316**,** 1271-5.
HOURIHANE, J. O., SMITH, P. K. & STROBEL, S. 2002. Food allergy in children. *Indian J Pediatr,* 69**,** 61-7.
HUANG, X., LIN, J. & DEMNER-FUSHMAN, D. 2006. Evaluation of PICO as a knowledge representation for clinical questions. *AMIA Annu Symp Proc***,** 359-63.
HURLBURT, B. K., OFFERMANN, L. R., MCBRIDE, J. K., MAJOREK, K. A., MALEKI, S. J. & CHRUSZCZ, M. 2013. Structure and function of the peanut panallergen Ara h 8. *J Biol Chem,* 288**,** 36890-901.
IRVINE, A. D. & MCLEAN, W. H. 2006. Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. *J Invest Dermatol,* 126**,** 1200-2.
IRVINE, A. D., MCLEAN, W. H. & LEUNG, D. Y. 2011. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med,* 365**,** 1315-27.
IWEALA, O. I. & BURKS, A. W. 2016. Food Allergy: Our Evolving Understanding of Its Pathogenesis, Prevention, and Treatment. *Curr Allergy Asthma Rep,* 16**,** 37.
JONES, S. M., PONS, L., ROBERTS, J. L., SCURLOCK, A. M., PERRY, T. T., KULIS, M., SHREFFLER, W. G., STEELE, P., HENRY, K. A., ADAIR, M., FRANCIS, J. M., DURHAM, S., VICKERY, B. P., ZHONG, X. & BURKS, A. W. 2009. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol,* 124**,** 292-300, 300 e1-97.
KEET, C. A., JOHNSON, K., SAVAGE, J. H., HAMILTON, R. G. & WOOD, R. A. 2013. Evaluation of Ara h2 IgE thresholds in the diagnosis of peanut allergy in a clinical population. *J Allergy Clin Immunol Pract,* 1**,** 101-3.
KIM, E. H., BIRD, J. A., KULIS, M., LAUBACH, S., PONS, L., SHREFFLER, W., STEELE, P., KAMILARIS, J., VICKERY, B. & BURKS, A. W. 2011. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol,* 127**,** 640-6 e1.
KIM, H., HAN, Y., KIM, K., LEE, J.Y., KIM, M., AHN, K., KIM, J. 2016. Diagnostic value of specific IgE to peanut and Ara h 2 in Korean children with peanut allergy. *Allergy Asthma Immunol Res,* 8**,** 156-160.
KIM, J. S. & NOWAK-WEGRZYN, A. 2011. Component-Resolved Diagnostics: Shedding Light on the So-Called 'Squishy Science' of Food Allergies? *International Archives of Allergy and Immunology,* 156.
KLEBER-JANKE, T., CRAMERI, R., APPENZELLER, U., SCHLAAK, M., BECKER, W.M. 1999. Selective cloning of peanut allergens, including profilin and @S albumins, by phage display technology. *Int Arch Allergy Immunol,* 119.
KLEMANS, R. J., OS-MEDENSORP, H., BLANKESTIJN., M., BRUIJNZEEL-KOOMEN, C.A.F.M., JNOL, E.F., KNULST, A.C. 2015. Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review. *Clin Exp Allergy,* 45**,** 720-730.
KLEMANS, R. J., VAN OS-MEDENDORP, H., BLANKESTIJN, M., BRUIJNZEEL-KOOMEN, C. A., KNOL, E. F. & KNULST, A. C. 2015. Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review. *Clin Exp Allergy,* 45**,** 720-30.
KLEMANS, R. J. B., OTTE, D., KNOL, M., KNOL, E., MEIJER, Y., FRITS, H.J., GMELIG-MYLING, C.A.F.M., BRUIJNZEEL-KOOMEN, A.C., PASMANS, S.G.M.A. 2013. The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic predicted model. *Journal of Allergy and Clinical Immunology,* 131**,** 157-63.
KLINNERT, M. D. & ROBINSON, J. L. 2008. Addressing the psychological needs of families of food-allergic children. *Curr Allergy Asthma Rep,* 8**,** 195-200.
KOPPELMAN, S. J. 1999. Heat-induced conformational changes of Ara h 1, a major peanut allergen, do not affect its allergenic properties. *Journal of Biological Chemistry,* 274**,** 4770-4777.

KOPPELMAN, S. J., DE JONG, G. A. H., LAAPER-ERTMANN, M., PEETERS, K. A. B. M., KNULST, A. C., HEFLE, S. L. & KNOL, E. F. 2005. Purification and immunoglobulin E-binding properties of peanut allergen Ara h 6: evidence for cross-reactivity with Ara h 2. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology,* 35**,** 490-7.

KOPPELMAN, S. J., HEFLE, S. L., TAYLOR, S. L. & DE JONG, G. A. H. 2010. Digestion of peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6: A comparative in vitro study and partial characterization of digestion-resistant peptides. *Molecular Nutrition & Food Research,* 54.

KOPPELMAN, S. J., KNOL, E. F., VLOOSWIJK, R. A., WENSING, M., KNULST, A. C., HEFLE, S. L., GRUPPEN, H. & PIERSMA, S. 2003. Peanut allergen Ara h 3: isolation from peanuts and biochemical characterization. *Allergy,* 58**,** 1144-51.

KOPPELMAN, S. J., VLOOSWIJK, R. A., KNIPPELS, L. M., HESSING, M., KNOL, E. F., VAN REIJSEN, F. C. & BRUIJNZEEL-KOOMEN, C. A. 2001. Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. *Allergy,* 56**,** 132-7.

KOPPELMAN, S. J., WENSING, M., ERTMANN, M., KNULST, A.C., KNOL, E.F. 2004. Relevance of Ara h1, Ara h2 and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen. *Clin Exp Allergy,* 34**,** 583-590.

KRAUSE, S., REESE, G., RANDOW, S., ZENNARO, D., QUARATINO, D., PALAZZO, P., CIARDIELLO, M. A., PETERSEN, A., BECKER, W. M. & MARI, A. 2009. Lipid transfer protein (Ara h 9) as a new peanut allergen relevant for a Mediterranean allergic population. *J Allergy Clin Immunol,* 124**,** 771-8.e5.

KUKKONEN, A. K., PELKONEN, A. S., MAKINEN-KILJUNEN, S., VOUTILAINEN, H. & MAKELA, M. J. 2015. Ara h 2 and Ara 6 are the best predictors of severe peanut allergy: a double-blind placebo-controlled study. *Allergy,* 70**,** 1239-45.

KUZNIAK, P. 2006. The mast cell degranulation process.

LACK, G., FOX, D., NORTHSTONE, K. & GOLDING, J. 2003a. Factors associated with the development of peanut allergy in childhood. *N Engl J Med,* 348**,** 977-85.

LACK, G., FOX, D., NORTHSTONE, K., GOLDING, J. & TEAM, A. L. S. O. P. A. C. S. 2003b. Factors associated with the development of peanut allergy in childhood. *N Engl J Med,* 348**,** 977-85.

LANGE, L., BEYER, K. & KLEINE-TEBBE, J. 2014. Benefits and limitations of molecular diagnostics in peanut allergy: Part 14 of the series Molecular Allergology. *Allergo J Int,* 23**,** 158-163.

LAUER, I., DUERINGER, N., POKOJ, S., REHM, S., ZOCCATELLI, G., REESE, G., MIGUEL-MONCIN, M. S., CISTERO-BAHIMA, A., ENRIQUE, E., LIDHOLM, J., VIETHS, S. & SCHEURER, S. 2009. The non-specific lipid transfer protein, Ara h 9, is an important allergen in peanut. *Clin Exp Allergy,* 39**,** 1427-37.

LAVINE, E., CLARKE, A., JOSEPH, L., SHAND, G., ALIZADEHFAR, R., ASAI, Y., CHAN, E. S., HARADA, L., ALLEN, M. & BEN-SHOSHAN, M. 2015. Peanut avoidance and peanut allergy diagnosis in siblings of peanut allergic children. *Clin Exp Allergy,* 45**,** 249-54.

LEBOVIDGE, J. S., STRAUCH, H., KALISH, L. A. & SCHNEIDER, L. C. 2009. Assessment of psychological distress among children and adolescents with food allergy. *J Allergy Clin Immunol,* 124**,** 1282-8.

LEHMANN, K., SCHWEIMER, K., REESE, G., RANDOW, S., SUHR, M., BECKER, W. M., VIETHS, S. & ROSCH, P. 2006. Structure and stability of 2S albumin-type peanut allergens: implications for the severity of peanut allergic reactions. *Biochem J,* 395**,** 463-72.

LEO, S. H., DEAN, J. M., JUNG, B., KUZELJEVIC, B. & CHAN, E. S. 2015. Utility of Ara h 2 sIgE levels to predict peanut allergy in Canadian children. *J Allergy Clin Immunol Pract,* 3**,** 968-9.

LIEBERMAN, J. A., GLAUMANN, S., BATELSON, S., BORRES, M.P., SAMPSON, H.A., NILSSON, C. 2015. The utility of peanut components in the diagnosis of IgE-mediated peanut allergy among distinct populations. *J Allergy Clin Immunol,* 1**,** 75-82.

LOPES DE OLIVEIRA, L. C., ADERHOLD, M., BRILL, M., SCHULZ, G., ROLINCK-WERNINGHAUS, C., MILLS, C., NIGGEMAN, B., NASPITZ, C.K., WAHN, U., BEYER, K. 2013. The value of specific IgE to peanut and its component Ara h 2 in the diagnosis of peanut allergy. *Allergy Clin Immunol Pract,* 1**,** 394-8.

MACDOUGALL, C. F., CANT, A. J. & COLVER, A. F. 2002. How dangerous is food allergy in childhood? The incidence of severe and fatal allergic reactions across the UK and Ireland. *Arch Dis Child,* 86**,** 236-9.

MARTINET, J., COUDERC, L., RENOSI, F., BOBEE, V., MARGUET, C. & BOYER, O. 2016. Diagnostic Value of Antigen-Specific Immunoglobulin E Immunoassays against Ara h 2 and Ara h 8 Peanut Components in Child Food Allergy. *Int Arch Allergy Immunol,* 169**,** 216-22.

MATSUO, H., YOKOOJI, T. & TAOGOSHI, T. 2015. Common food allergens and their IgE-binding epitopes. *Allergol Int,* 64**,** 332-43.

MCMURRY, L. O. 1982. *George Washington Carver: Scientist and Symbol,* Oxford, UK., Oxford University Press.

MITTAG, D., AKKERDAAS, J., BALLMER-WEBER, B. K., VOGEL, L., WENSING, M., BECKER, W. M., KOPPELMAN, S. J., KNULST, A. C., HELBLING, A., HEFLE, S. L., VAN REE, R. & VIETHS, S. 2004. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. *J Allergy Clin Immunol,* 114**,** 1410-7.

MORENO, F. J. 2007. Gastrointestinal digestion of food allergens: effect on their allergenicity. *Biomed Pharmacother,* 61**,** 50-60.

MOSER, M. & LEO, O. 2010. Key concepts in immunology. *Vaccine,* 28 Suppl 3**,** C2-13.

MOVERARE, R., AHLSTEDT, S., BENGTSSON, U., BORRES, M. P., VAN HAGE, M., POORAFSHAR, M., SJOLANDER, S., AKERSTROM, J. & VAN ODIJK, J. 2011. Evaluation of IgE Antibodies to Recombinant Peanut Allergens in Patients with Reported Reactions to Peanut. *International Archives of Allergy and Immunology,* 156.

MUELLER, G. A., GOSAVI, R. A., POMES, A., WUENSCHMANN, S., MOON, A. F., LONDON, R. E. & PEDERSEN, L. C. 2011. Ara h 2: crystal structure and IgE binding distinguish two subpopulations of peanut allergic patients by epitope diversity. *Allergy,* 66.

MUELLER, H. L. 1959. Insect allergy. *Pediatr Clin North Am,* 6**,** 917-952.

MURARO, A., AGACHE, I., CLARK, A., SHEIKH, A., ROBERTS, G., AKDIS, C. A., BORREGO, L. M., HIGGS, J., HOURIHANE, J. O., JORGENSEN, P., MAZON, A., PARMIGIANI, D., SAID, M., SCHNADT, S., VAN OS-MEDENDORP, H., VLIEG-BOERSTRA, B. J., WICKMAN, M., EUROPEAN ACADEMY OF, A. & CLINICAL, I. 2014. EAACI food allergy and anaphylaxis guidelines: managing patients with food allergy in the community. *Allergy,* 69**,** 1046-57.

MURARO, A., CLARK, A., BEYER, K., BORREGO, L. M., BORRES, M., LODRUP CARLSEN, K. C., CARRER, P., MAZON, A., RANCE, F., VALOVIRTA, E., WICKMAN, M. & ZANCHETTI, M. 2010. The management of the allergic child at school: EAACI/GA2LEN Task Force on the allergic child at school. *Allergy,* 65**,** 681-9.

NAGLER-ANDERSON, C. 2001. Man the barrier! Strategic defences in the intestinal mucosa. *Nat Rev Immunol,* 1**,** 59-67.

NAMORK, E. & STENSBY, B. A. 2015. Peanut sensitization pattern in Norwegian children and adults with specific IgE to peanut show age related differences. *Allergy Asthma Clin Immunol,* 11**,** 32.
NICOLAOU, N. & CUSTOVIC, A. 2011. Molecular diagnosis of peanut and legume allergy. *Curr Opin Allergy Clin Immunol,* 11**,** 222-8.
NICOLAOU, N., MURRAY, C., BELGRAVE, D., POORAFSHAR, M., SIMPSON, A. & CUSTOVIC, A. 2011. Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. *J Allergy Clin Immunol,* 127**,** 684-5.
NICOLAOU, N., POORAFSHAR, M., MURRAY, C., SIMPSON, A., WINELL, H., KERRY, G., HARLIN, A., WOODCOCK, A., AHLSTEDT, S. & CUSTOVIC, A. 2010a. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *The Journal of allergy and clinical immunology,* 125**,** 191-7.e1-13.
NICOLAOU, N., POORAFSHAR, M., MURRAY, C., SIMPSON, A., WINELL, H., KERRY, G., HRLIN, A., WOODCOCK, A., AHLSTEDT, S. & CUSTOVIC, A. 2010b. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol,* 125**,** 191-7.e1-13.
NOLAN, R. C., RICHMOND, P., PRESCOTT, S. L., MALLON, D. F., GONG, G., FRANZMANN, A. M., NAIDOO, R. & LOH, R. K. 2007. Skin prick testing predicts peanut challenge outcome in previously allergic or sensitized children with low serum peanut-specific IgE antibody concentration. *Pediatr Allergy Immunol,* 18**,** 224-30.
NOSBAUM, A., VOCANSON, M., ROZIERES, A., HENNINO, A. & NICOLAS, J. F. 2009. Allergic and irritant contact dermatitis. *Eur J Dermatol,* 19**,** 325-32.
NOWAK-WEGRZYN, A., ASSA'AD, A. H., BAHNA, S. L., BOCK, S. A., SICHERER, S. H. & TEUBER, S. S. 2009. Work Group report: oral food challenge testing. *J Allergy Clin Immunol,* 123**,** S365-83.
NWARU, B. I., HICKSTEIN, L., PANESAR, S. S., MURARO, A., WERFEL, T., CARDONA, V., DUBOIS, A. E., HALKEN, S., HOFFMANN-SOMMERGRUBER, K., POULSEN, L. K., ROBERTS, G., VAN REE, R., VLIEG-BOERSTRA, B. J., SHEIKH, A., ALLERGY, E. F. & ANAPHYLAXIS GUIDELINES, G. 2014. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy,* 69**,** 62-75.
OYOSHI, M. K., MURPHY, G.F., GEHA, R.S. 2009. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein agent. *Journal of Allergy and Clinical Immunology,* 124.
PALMER, C. N., IRVINE, A. D., TERRON-KWIATKOWSKI, A., ZHAO, Y., LIAO, H., LEE, S. P., GOUDIE, D. R., SANDILANDS, A., CAMPBELL, L. E., SMITH, F. J., O'REGAN, G. M., WATSON, R. M., CECIL, J. E., BALE, S. J., COMPTON, J. G., DIGIOVANNA, J. J., FLECKMAN, P., LEWIS-JONES, S., ARSECULERATNE, G., SERGEANT, A., MUNRO, C. S., EL HOUATE, B., MCELREAVEY, K., HALKJAER, L. B., BISGAARD, H., MUKHOPADHYAY, S. & MCLEAN, W. H. 2006. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet,* 38**,** 441-6.
PALMER, G. W., DIBBERN, D. A., JR., BURKS, A. W., BANNON, G. A., BOCK, S. A., PORTERFIELD, H. S., MCDERMOTT, R. A. & DRESKIN, S. C. 2005. Comparative potency of Ara h 1 and Ara h 2 in immunochemical and functional assays of allergenicity. *Clinical immunology (Orlando, Fla.),* 115**,** 302-12.
PALMER, G. W., DIBBERN, D. A., BURKS, A.W., VANNON, G.A., BOCK, S.A., PORTERFIELD, H.S., MCDERMOTT, R.A., DRESKIN, S.C. 2002. Ara h 2 is greater than 50 times more potent than Ara h 1 in a functional assay of peanut

protein allergenicity, a difference not appreciated with immunoblots. *J Allergy Clin Immunol,* 109**,** S300.
PARHAM, P. 2009. *The immune system,* Abingdon, UK, Garland Science, Taylor & Francis Group.
PARKIN, J. & COHEN, B. 2001. An overview of the immune system. *Lancet,* 357**,** 1777-89.
PEDROSA, M., BOYANO-MARTINEZ, T., GARCIA-ARA, M. C., CABALLERO, T. & QUIRCE, S. 2012. Peanut seed storage proteins are responsible for clinical reactivity in Spanish peanut-allergic children. *Pediatr Allergy Immunol,* 23**,** 654-9.
PELE, M. 2010. Peanut allergens. *Romanian Biotechnological Letters,* 15**,** 5204-5212.
PERKIN, M. R., LOGAN, K., MARRS, T., RADULOVIC, S., CRAVEN, J., FLOHR, C., LACK, G. & TEAM, E. A. T. S. 2016. Enquiring About Tolerance (EAT) study: Feasibility of an early allergenic food introduction regimen. *J Allergy Clin Immunol,* 137**,** 1477-1486 e8.
PETERS, R. L., ALLEN, K. J., DHARMAGE, S. C., KOPLIN, J. J., DANG, T., TILBROOK, K. P., LOWE, A., TANG, M. L., GURRIN, L. C. & HEALTHNUTS, S. 2015. Natural history of peanut allergy and predictors of resolution in the first 4 years of life: A population-based assessment. *J Allergy Clin Immunol,* 135**,** 1257-66 e1-2.
PETERS, R. L., ALLEN, K. J., DHARMAGE, S. C., TANG, M. L., KOPLIN, J. J., PONSONBY, A. L., LOWE, A. J., HILL, D., GURRIN, L. C. & HEALTHNUTS, S. 2013. Skin prick test responses and allergen-specific IgE levels as predictors of peanut, egg, and sesame allergy in infants. *J Allergy Clin Immunol,* 132**,** 874-80.
PETERSEN, A., KULL, S., RENNERT, S., BECKER, W. M., KRAUSE, S., ERNST, M., GUTSMANN, T., BAUER, J., LINDNER, B. & JAPPE, U. 2015. Peanut defensins: Novel allergens isolated from lipophilic peanut extract. *J Allergy Clin Immunol,* 136**,** 1295-301 e1-5.
PHADIA. 2014. *ImmunoCAP Specific IgE Fluoroenzyneimmunoassay Directions for Use* [Online]. Uppsala, Sweden. Available: http://www.dfu.phadia.com/Data/Pdf/56cb2b6389c23251d0d2b2ff.pdf [Accessed 19/07/2016 2016].
PONS, L., CHERY, C., ROMANO, A., NAMOUR, F., ARTESANI, M. C. & GUEANT, J. L. 2002. The 18 kDa peanut oleosin is a candidate allergen for IgE-mediated reactions to peanuts. *Allergy,* 57 Suppl 72**,** 88-93.
POST, P. O. O. S. A. T. 2004. Vaccines and Public Health. *POSTnote.* London: POST.
PYRHONEN, K., NAYHA, S., KAILA, M., HILTUNEN, L. & LAARA, E. 2009. Occurrence of parent-reported food hypersensitivities and food allergies among children aged 1-4 yr. *Pediatr Allergy Immunol,* 20**,** 328-38.
QUADAS-2. 2016. Available: http;//http://www.bristol.ac.uk/social-community-medicine/projects/quadas/quadas-2/.
RANCE, F. 2002. [Skin tests for the diagnosis of food allergy]. *Rev Mal Respir,* 19**,** 258-9.
RANCE, F., ABBAL, M. & LAUWERS-CANCES, V. 2002. Improved screening for peanut allergy by the combined use of skin prick tests and specific IgE assays. *J Allergy Clin Immunol,* 109**,** 1027-33.
RCP, R. C. O. P. 2003. Allergy: the unmet need. A blueprint for better patient care. A report of the Royal College of Physicians Working Party on the Provision of Allergy Services in the UK. London: Royal College of Physicians.
REMINGTON, B. C., BAUMERT, J. L., BLOM, W. M., HOUBEN, G. F., TAYLOR, S. L. & KRUIZINGA, A. G. 2015. Unintended allergens in precautionary labelled and unlabelled products pose significant risks to UK allergic consumers. *Allergy,* 70**,** 813-9.
ROBERTS, G. & LACK, G. 2005. Diagnosing peanut allergy with skin prick and specific IgE testing. *J Allergy Clin Immunol,* 115**,** 1291-6.

ROBERTS, G., LACK., G. 2005. Diagnosing peanut allergy with skin prick and specific IgE testing. *J Allergy Clin Immunol,* 115.
SAMPSON, H. A. 1999. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol,* 103**,** 717-28.
SAMPSON, H. A. 2001. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol,* 107**,** 891-6.
SAMPSON, H. A. & HO, D. G. 1997. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol,* 100**,** 444-51.
SAUER, J. D. 1993. *Historical geography of crop plants: A select roster,* New York, CRC Press.
SAVAGE, J. H., MATSUI, E. C., SKRIPAK, J. M. & WOOD, R. A. 2007. The natural history of egg allergy. *J Allergy Clin Immunol,* 120**,** 1413-7.
SCURLOCK, A. M. & BURKS, A. W. 2004. Peanut allergenicity. *Ann Allergy Asthma Immunol,* 93**,** S12-8.
SCURRAN15 2015. Process of denaturation. *In:* DENATURATION.SVG, P. O. (ed.).
SEN, M. 2002. Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. *Journal of Immunology,* 169**,** 882-887.
SHEWRY, P. R., NAPIER, J. A. & TATHAM, A. S. 1995. Seed storage proteins: structures and biosynthesis. *Plant Cell,* 7**,** 945-56.
SHREFFLER, W. G., BEYER, K., CHU, T.-H. T., BURKS, A. W. & SAMPSON, H. A. 2004. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *The Journal of allergy and clinical immunology,* 113**,** 776-82.
SICHERER, S. 1998. Clinical features of acute allergic reactions to peanuts and tree nuts in children. *Pediatrics,* 102**,** e6.
SICHERER, S. 2002. Food allergy. *The Lancet,* 360**,** 701-10.
SICHERER, S. H., MUNOZ-FURLONG, A., GODBOLD, J. H. & SAMPSON, H. A. 2010. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J Allergy Clin Immunol,* 125**,** 1322-6.
SICHERER, S. H. & SAMPSON, H. A. 2010. Food allergy. *J Allergy Clin Immunol,* 125**,** S116-25.
SICHERER, S. H., WOOD, R. A., VICKERY, B. P., JONES, S. M., LIU, A. H., FLEISCHER, D. M., DAWSON, P., MAYER, L., BURKS, A. W., GRISHIN, A., STABLEIN, D. & SAMPSON, H. A. 2014. The natural history of egg allergy in an observational cohort. *J Allergy Clin Immunol,* 133**,** 492-9.
SINGH B, S. U. 1991. Peanut as a source of protein for human foods. *Plant Foods Hum Nutr,* 41**,** 165-77.
SISKIYOUS, C. O. T. 2010. *Protein denaturation* [Online]. California, US. Available: http://www.yellowtang.org/images/protein_denaturatio_c_la_784.jpg 2010].
SKOLNICK, H. S., CONOVER-WALKER, M.K., KOERNER, C.B., SAMPSON, H.A., BURKS, W., WOOD, R.A. 2001. The natural history of peanut allergy. *J Allergy Clin Immunol,* 107**,** 367-74.
SPERGEL, J. M., BOGUNIEWICZ, M., SCHNEIDER, L., HANIFIN, J. M., PALLER, A. S. & EICHENFIELD, L. F. 2015. Food Allergy in Infants With Atopic Dermatitis: Limitations of Food-Specific IgE Measurements. *Pediatrics,* 136**,** e1530-8.
SPORIK, R., HILL, D. J. & HOSKING, C. S. 2000. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy,* 30**,** 1540-6.
STANLEY, J. S., BANNON, G.A. 1999. Biochemistry of food allergens. *Clin Rev Allergy Immunol,* 17**,** 279-91.
SUHR, M., WICKLEIN, D., LEPP, U. & BECKER, W.-M. 2004. Isolation and characterization of natural Ara h 6: evidence for a further peanut allergen with putative clinical relevance based on resistance to pepsin digestion and heat. *Molecular nutrition & food research,* 48**,** 390-9.

SURTANNON, N., NGAMPHAIBOON, J., WONGPIYABOVORN, J., PURIPOKAI, P., CHATCHATEE, P. 2013. Component-resolved diagnostics for the evaluation of peanut allergy in a low prevalence area. *Pediatr Allergy Immunol,* 24**,** 665-670.

SWAIN, S. L., BRADLEY, L. M., CROFT, M., TONKONOGY, S., ATKINS, G., WEINBERG, A. D., DUNCAN, D. D., HEDRICK, S. M., DUTTON, R. W. & HUSTON, G. 1991. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol Rev,* 123**,** 115-44.

TARIQ, S. M., STEVENS, M., MATTHEWS, S., RIDOUT, S., TWISELTON, R. & HIDE, D. W. 1996. Cohort study of peanut and tree nut sensitisation by age of 4 years. *Bmj,* 313**,** 514-7.

TAYLOR, S. L., CREVEL, R. W., SHEFFIELD, D., KABOUREK, J. & BAUMERT, J. 2009. Threshold dose for peanut: risk characterization based upon published results from challenges of peanut-allergic individuals. *Food Chem Toxicol,* 47**,** 1198-204.

TAYLOR, S. L., LEHRER, S.B. 1996. Principles and characteristics of food allergens. *Crit Rev Food Sci Nutr,* 36**,** S91-118.

TRENDELENBURG, V., ROHRBACH, A., SCHULZ, G., SCHWARZ, V. & BEYER, K. 2014. Molecular sIgE profile in infants and young children with peanut sensitization and eczema. *Allergo J Int,* 23**,** 152-157.

TUANO, K. S. & DAVIS, C. M. 2015. Utility of Component-Resolved Diagnostics in Food Allergy. *Curr Allergy Asthma Rep,* 15**,** 32.

VALENTA, R., TWAROCH, T. & SWOBODA, I. 2007. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. *J Investig Allergol Clin Immunol,* 17 Suppl 1**,** 36-40.

VAN BOXTEL, E. L., VAN BEERS, M. M., KOPPELMAN, S. J., VAN DEN BROEK, L. A. & GRUPPEN, H. 2006. Allergen Ara h 1 occurs in peanuts as a large oligomer rather than as a trimer. *J Agric Food Chem,* 54**,** 7180-6.

VAN ERP, F. C., KLEMANS, R. J., MEIJER, Y., VAN DER ENT, C. K. & KNULST, A. C. 2016. Using Component-Resolved Diagnostics in the Management of Peanut-Allergic Patients. *Curr Treat Options Allergy,* 3**,** 169-180.

VAN VEEN, L. N., HERON, M., BATSTRA, M., VAN HAARD, P. M. & DE GROOT, H. 2016. The diagnostic value of component-resolved diagnostics in peanut allergy in children attending a Regional Paediatric Allergology Clinic. *BMC Pediatr,* 16**,** 74.

VENTER, C., HASAN ARSHAD, S., GRUNDY, J., PEREIRA, B., BERNIE CLAYTON, C., VOIGT, K., HIGGINS, B. & DEAN, T. 2010. Time trends in the prevalence of peanut allergy: three cohorts of children from the same geographical location in the UK. *Allergy,* 65**,** 103-8.

VEREDA, A., VAN HAGE, M., AHLSTEDT, S., IBAEZ, M. D., CUESTA-HERRANZ, J., VAN ODIJK, J., WICKMAN, M. & SAMPSON, H. A. 2011. Peanut allergy: Clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol,* 127**,** 603-7.

WAINSTEIN, B. K., YEE, A., JELLEY, D., ZIEGLER, M. & ZIEGLER, J. B. 2007. Combining skin prick, immediate skin application and specific-IgE testing in the diagnosis of peanut allergy in children. *Pediatr Allergy Immunol,* 18**,** 231-9.

WEIDINGER, S., ILLIG, T.,BAURECHT, H., IRVINE, A.D., RODRIGUEZ, E., DIAZ-LACAVA, A., KLOPP, N., WAGENPFELL, S., ZHAO, Y., LIAO, H., LEE, S.P., PALMER, C.N., JENNECK, C., MAINTZ, L. HAGENMANN, T., BEHRENDT, H., RING, J., NOTHEN, M.M., MCLEAN, W.H., NOVAK, N. 2006. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *Journal of Allergy and Clinical Immunology,* 118**,** 214-9.

WEIDINGER, S., O'SULLIVAN, M., ILLIG, T., BAURECHT, H., DEPNER, M., RODRIGUEZ, E., RUETHER, A., KLOPP, N., VOGELBERG, C., WEILAND, S. K., MCLEAN, W. H., VON MUTIUS, E., IRVINE, A. D. & KABESCH, M. 2008. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol,* 121**,** 1203-1209 e1.

WENNERGREN, G. 2009. What if it is the other way around? Early introduction of peanut and fish seems to be better than avoidance. *Acta Paediatr,* 98**,** 1085-7.
WHITING, P. F., RUTJES, A.W.S., WESTWOOD, M.E., MALLETT, S., DEEKS, J.J., REITSMA, J.B., LEEFLANG, M.M., STERNE, J.A.C., BOSSUYT, P.M.M. 2011. QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med,* 155**,** 529-536.
ZHOU, Y., WANG, J. S., YANG, X. J., LIN, D. H., GAO, Y. F., SU, Y. J., YANG, S., ZHANG, Y. J. & ZHENG, J. J. 2013. Peanut Allergy, Allergen Composition, and Methods of Reducing Allergenicity: A Review. *Int J Food Sci,* 2013**,** 909140.

Bibliography

CAMPBELL, M.J. & MACHIN, D. Medical Statistics: A Commonsense Approach. 2nd edition. John Wiley & Sons, Chichester, 1993.

HOLGATE, S.T., CHURCH, M.K., LICHTENSTEIN, L.M. Allergy (3rd Edition). Mosby, Elsevier, Philadelphia, 2006.

KUZNIAR, P. 2006. The mast cell degranulation process. Wikimedia commons. Web 1 March 2016.
http://upload.wikimedia.og/wikipedia/commons/thumb/3/36/Allergy_degranulation_process

FIELD, A. Discovering statistics using IBM SPSS statistics (4th Edition). Sage, London, 2013.

Appendices

List of Appendices

Appendix 1
Parent Information Sheet

Parent Information Sheet v4.3

The NutCracker Study
Parent Information Sheet

The NutCracker Study: A study of egg allergic children with positive allergy tests to peanut - can a single blood test to a peanut protein called Ara h 2 predict which egg allergic children will also have peanut allergy?

Researchers: Deb Marriage, Dr John Henderson & Dr Huw Thomas

Staff in the Allergy Clinic at Bristol Children's Hospital would like to invite you to participate in a research study called 'The NutCracker Study' Project. Before you decide whether to take part, it is important for you to understand why the research project is being done and what it would involve. Pease take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything which is not clear, or if you would like further information.

What is the purpose of this study?
Early research in other centres has suggested that a particular peanut protein called 'Ara h 2' may be helpful in predicting whether a child has peanut allergy or not. We would like to know if performing an allergy test to this protein, Ara h 2, may be more accurate that testing children to whole peanut, which is what we do at the moment.

Why has my child been invited to participate?
Your child has been sent this invitation letter and information sheet because they are attending our allergy clinic with egg allergy. Peanut allergy is more common in children with egg allergy and so if your child has never eaten peanuts, they will also routinely have allergy testing for whole peanut at this clinic appointment. If your child has a positive skin prick test to whole peanut in clinic, they will then be eligible for this study.

Do we have to take part?
It is entirely up to you to decide if you would like your child to take part in this study. If you do decide to take part you will be asked to sign a consent form. Should you decide to take part, you are still free to change your mind and withdraw from the study at any time without giving a reason. Your decision to participate will not affect your child's medical care.

What will happen if we take part?
With your consent your child will be offered a blood test to Ara h 2 (a peanut protein). Your child will already be having a routine blood test where 5 millilitres of blood is taken (an amount that will fit on a teaspoon). So for this extra test to Ara h 2, we will only need to take an extra 0.5 millilitres of blood which we can during the routine blood test. There is no need to use another needle and your child will only have a single blood test.

If your child has a positive skin prick test or blood test to peanut, your child's doctor will refer your child to the day care unit for an oral peanut provocation challenge. This is to see if they can eat peanut without having an allergic reaction. At present, this is the only way we have of telling if your child definitely has peanut allergy. The results of this challenge will be sent to the staff involved in the 'The NutCracker Study project. They will look to see if there is a difference between Ara h 2 allergy levels in children who pass a peanut challenge without reacting and those who don't.

What are the possible advantages and risks of taking part?
We do not foresee any increased risks to your child by taking part in the study. Your child is already attending the clinic for a routine blood test and skin prick testing. In the present study, we will take a very small amount of extra blood and this poses no extra risk. As with all blood tests, some children who are scared of needles may faint, but the additional amount of blood will not increase this risk and we are used to making children as comfortable as possible if this happens.

What are the possible benefits of taking part?
If your child is peanut allergic then we will ensure that they are carrying the correct allergy medication and we will add the diagnosis of peanut allergy to their Allergy Action Plan, which they have at home and in school. We will also provide you with our general information sheet on having a peanut allergic child. However, the management of your child's peanut allergy is unlikely to change in the short-term as all of this is part of their routine clinical care which they would receive anyway after their peanut provocation challenge. The information resulting from this study would be used to shape our policy for the management of peanut allergy in the future within our hospital and possibly further afield. Ultimately we hope that this will benefit other children in the future.

What if something goes wrong?
We would not expect any harm to people from taking part in this study. If you are unhappy about any aspect of the way you or your child have been dealt with during this study, then the normal NHS complaints mechanisms will be available to you.

Will my taking part in the study be confidential?
The information collected in this study will be kept strictly confidential. All information will only be accessible by members of the research team. No individual names or details that would identify specific individuals will be included in the outputs of the research. All published and unpublished reports will not identify specific individuals.

What will happen to the results of the study?
The results will be analysed in two groups at the end of the study. After this we will be able to let you know what the final results have shown. The overall results of the study will be written up for publication in an academic journal. They will also be presented to other medical staff within the University Hospitals Bristol NHS Foundations Trust and presented at conferences.

Who is organising the research?
The study is being organised by Deborah Marriage, Lead Clinical Nurse Specialist in Asthma and Allergy and is supported by Dr John Henderson and Dr Huw Thomas, who are Consultant Chest Paediatricians. All members of staff are based at Bristol Royal Hospital for Children.

Who has reviewed the study?
The study proposal has been reviewed by the UHB Research and Development Department, and by the Camden and Islington Ethics Committee. It has also been reviewed by the Executive Research Officer at the University of Bath.

Remember that you can withdraw from the study at any time
Thank you for taking the time to read this. I hope that you and your child would like to participate.

Contact details:
If you would like to contact me at any point in the study my details are:

Deb Marriage
Lead Allergy Clinical Nurse Specialist
Room 101, The Dug-Out,
Lower Ground Floor
King David Building
Bristol Royal Hospital for Children
Upper Maudlin Street
Bristol BS2 8BJ

t: 0117 342 8248
e: deb.marriage@uhbristol.nhs.uk

Patient Support and Complaints Team(PALS)
University Hospitals Bristol
Welcome Centre
Queens Building
Bristol Royal Infirmary
Upper Maudlin Street
Bristol BS2 8HW

T: 0117 342 1050
e:pals@uhbristol.nhs.uk

Camden & Islington NRes Committee
t: 020 797 22545
e: nrescommittee.london-camdenandislington@nhs.net

Appendix 2
Child Information Sheet

Version 1.1
16.10.2014

What to do now…..

There is a form for your parents to sign to say that they agree it is ok for you to take part in the study.
The nurse, Deb looks after this form and she will ask your parents to sign it. There is also a form you can sign to say you would like to join in the study.

Any questions?

Do you want to know more about the project before you decide to take part? That's fine. Get in touch with us and we'll try to answer any questions you have. You can get in touch by phone, letter or email to:

The NutCracker *Study*
c/o Deb Marriage
Room 101, Lower Ground Floor, King David Building
Bristol Royal Hospital for Children
Upper Maudlin Street
Bristol BS2 8BJ

Telephone: 0117 342 8248
Email: deb.marriage@uhbristol.nhs.uk

University Hospitals Bristol NHS
NHS Foundation Trust

The NutCracker Study

Information leaflet for children and young people 7-11 years

Version 1.1
16.10.2014

The NutCracker *Study*

What's this all about?

The NutCracker Study is about finding a better way of being able to tell which children with egg allergy also have peanut allergy. We know that peanut allergy is very common in children with egg allergy. The study is about finding a better test that can tell us this, without actually having to feed children peanut to see if they are allergic to it.

What kind of new test?

The tests we use at the moment to predict peanut allergy are skin prick tests and blood tests. The new test will be a blood test which looks for different bits of peanut than those we usually test for.

Who are we?

Our names are Deb, Dr Huw Thomas and Professor John Henderson. You will meet one of us if you join in with the project. We are nurses and doctors who work in the Bristol Children's Hospital Allergy Clinics. These clinics are held at the Children's Hospital, Southmead Hospital or South Bristol Hospital.

What does taking part involve?

If you want to take part in The NutCracker Study one of us will take a tiny bit of extra blood when you your blood test in clinic. Then when you come into hospital in the future to see if you can eat peanut (called a 'peanut challenge'), one of us will record whether or not you have a reaction on that day.

It's private and confidential

This means that we will keep whatever you tell us secret from other people. We will not use your names in the project report. We would like to tell your family doctor that you are taking part in the study, if you are happy for us to do this.

Do you want to take part?

We hope that you will want to take part in the research but it is your choice. If you do not want to take part that is fine. If you decide to take part and later change your mind, that's ok too.

OTHER IMPORTANT INFORMATION:
Who else knows about the study?

The study outline has been closely looked at by the University of Bath, the Children's Hospital Research Team and the Camden and Islington Research Ethics Committee.

Who should I talk to if I am unhappy with the way the study has been run?
If you have a complaint, you should talk to the:

Patient Support and Complaints Team(PALS)
University Hospitals Bristol
Welcome Centre
Queens Building
Bristol Royal Infirmary
Upper Maudlin Street
Bristol BS2 8HW

t: 0117 342 1050
e:pals@uhbristol.nhs.uk

Appendix 3
Study Consent Form

University Hospitals Bristol NHS
NHS Foundation Trust

Centre Number:
Study Number:

Bristol Royal Hospital for Children
Paul O'Gorman Building
Bristol BS2 8BJ

CONSENT FORM v4.3

Title of Project: **The NutCracker Study: A study of egg allergic children with positive allergy tests to peanut - can a single blood test to a peanut protein called Ara h 2 predict which egg allergic children will have peanut allergy?**

Name of Lead Researcher: **Deborah Marriage**

Please initial box

1. I confirm that I have read and understand the information sheet (version 4.3) dated 10/12/2014 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my child's participation is voluntary and that I am free to withdraw from the study at any time, without giving any reason, without their medical care or legal rights being affected. ☐

3. I understand that relevant sections of any of my child's medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my child's participation in this research. I give permission for these individuals to have access to my child's records. ☐

4. I agree to my GP being informed of my child's participation in the study. ☐

5. I agree to my child taking part in the above study. ☐

_____	_____	_____
Name of Child	Date	Signature
_____	_____	_____
Name of Parent / Guardian	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

Appendix 4
GP Information Sheet

University Hospitals Bristol NHS
NHS Foundation Trust
Bristol Royal Hospital for Children
Paul O'Gorman Building
Upper Maudlin Street
Bristol
BS2 8BJ
Tel: 0117 923 0000

Dr
Dr Address

Date

Dear Dr,

RE: **Child Name:** **DOB:**

Child Address:

Participation in a research study

The NutCracker Study: A study of incidental sensitisation to peanut in egg allergic children, and the utility of component-resolved diagnostics in predicting clinical outcome.

Your patient is participating in the above research study which is investigating the value of specific IgE testing to the peanut component Ara h 2 in predicting peanut allergy in egg allergic infants and children. The results of your patient's specific IgE tests to peanut and Ara h 2 will be correlated with the outcome of their routine oral peanut challenge.

Please contact the research team if you require further information about this study.

Yours sincerely,

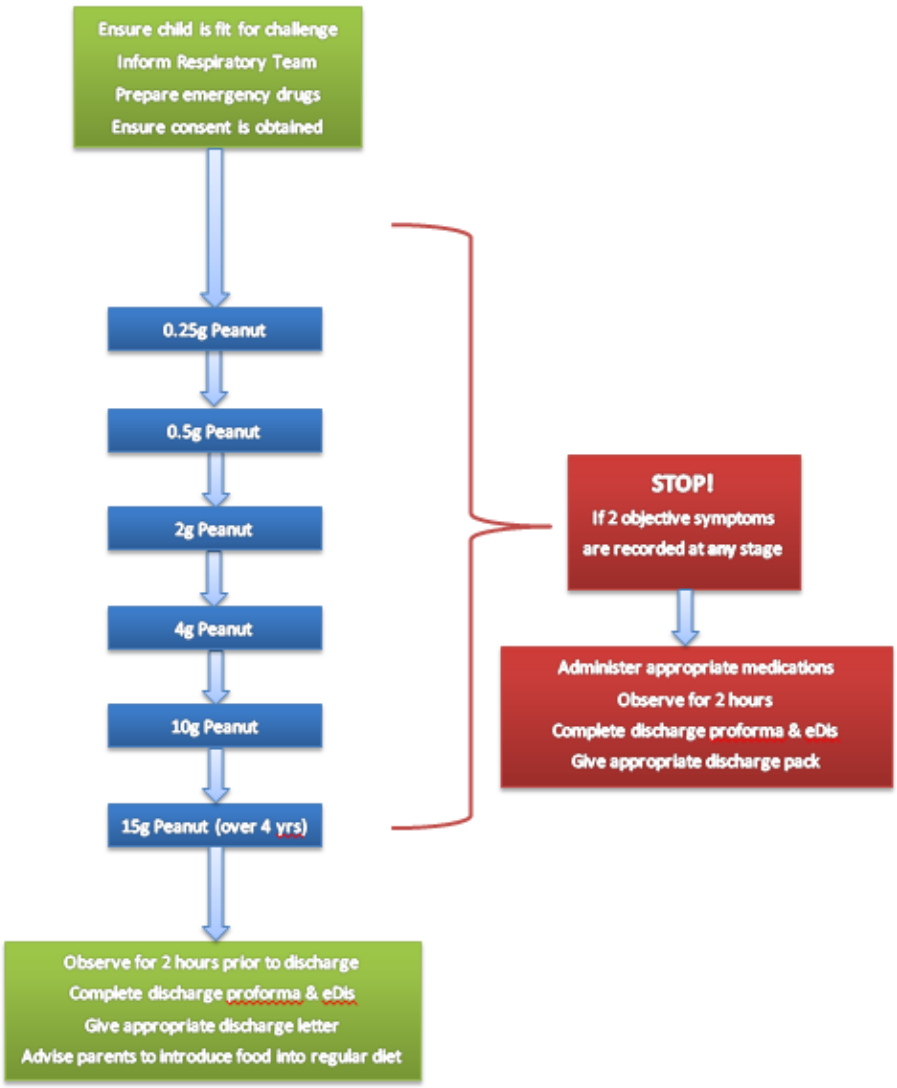
Deborah Marriage

Allergy and Respiratory Specialist Nurse and Chief Investigator
Direct Tel: 0117 342 8248
Email: deb.marriage@UHBristol.nhs.uk

Appendix 5
Challenge protocols

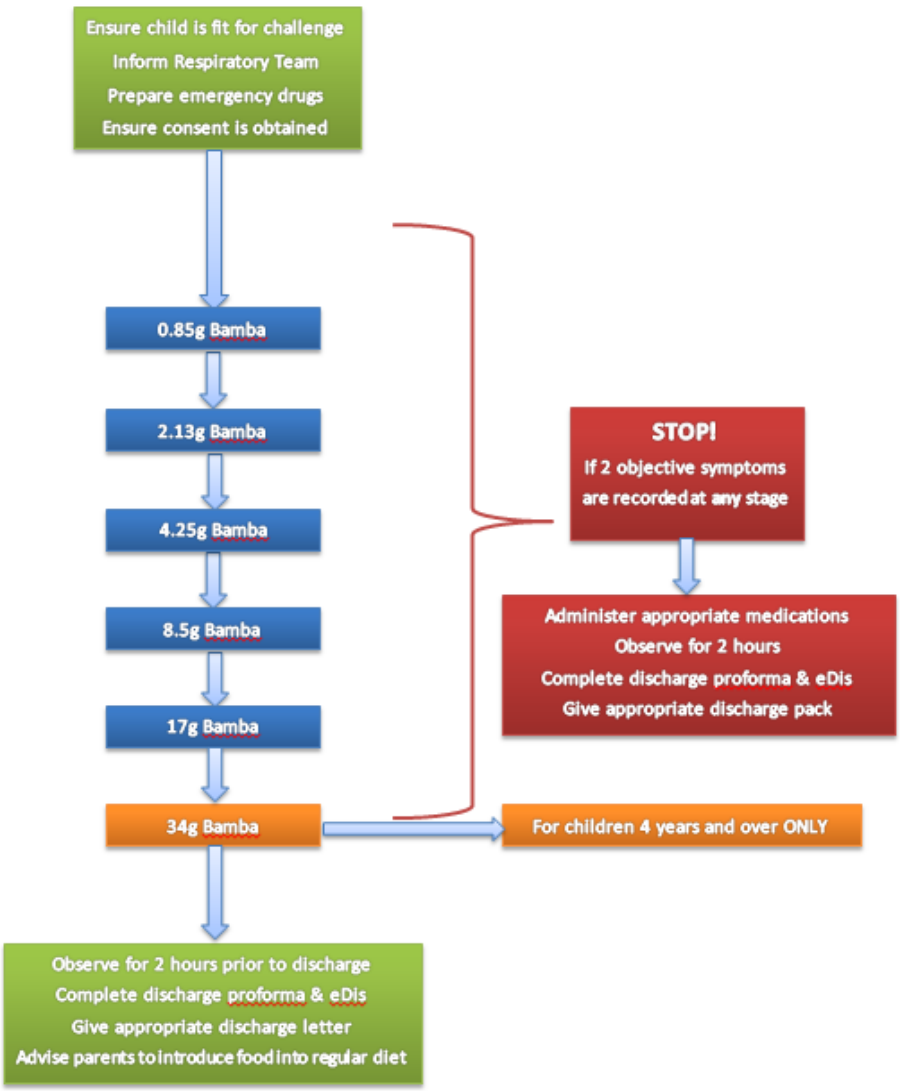
PEANUT CHALLENGE FLOWCHART

This flowchart must be used in conjunction with the Food Challenge Clinical Guideline and Symptom Score Sheet.



PEANUT BAMBA FLOWCHART

This flowchart must be used in conjunction with the Food Challenge Clinical Guideline and Symptom Score Sheet.



Appendix 6
Oral Challenge Symptom Score Sheet

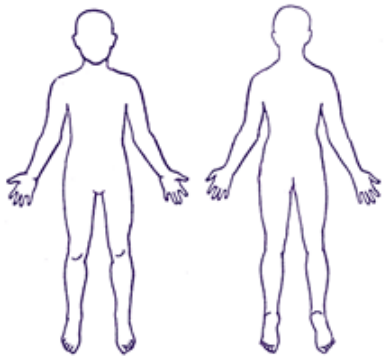
Oral Food Challenge Symptom Score Sheet

Possible reactions

I Skin

A Erythematous rash
% Area involved (____%)
(see body surface area diagram)

	Child	Infant <2
Head	4.5%	8.5%
Neck	1%	-
Anterior trunk	13%	13%
Posterior trunk	13%	13%
Leg	13%	14%
Arm	9%	9%



B Pruritus
0 = Absent
1 = Mild: occasional scratching
2 = Moderate: scratching continuously (for >2 minutes at a time)

C Urticaria/Angioedema
0 = Absent
1 = Mild: less than 3 hives
2 = Moderate: > 3 and <10 hives
3 = Severe: generalised involvement

D Rash
0 = Absent
1 = Mild: few areas of faint erythema
2 = Moderate: areas of erythema, macular & raised rash
3 = Severe: generalised marked erythema (>50%), extensive raised lesion (>25%) or vesiculation

II Upper Respiratory

A Sneezing / Itching
0 = Absent
1 = Mild: rare bursts
2 = Moderate: bursts <10, intermittent rubbing of nose / eyes / external ears canals
3 = Severe: continuous rubbing of nose / eyes, periocular swelling and / or long bursts of Sneezing

B Nasal Congestion
0 = Absent
1 = Mild: some hindrance to breathing
2 = Moderate: nostrils feel blocked, mostly breathing though mouth
3 = Severe: nostrils occluded

C Rhinorrhea
0 = Absent
1 = Mild: occasional sniffing
2 = Moderate: frequent sniffing, requires tissues
3 = Severe: nose runs freely despite sniffing and tissues

D Laryngeal
0 = Absent
1 = Mild: throat clearing, occasional cough
2 = Moderate: hoarseness, frequent dry cough
3 = Severe: inspiratory stridor

Modified from Bock SA, Sampson HA, Atkins FM et al. *Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual.* J Allergy Clin Immunol 1988;82:986-97.

III **Lower Respiratory**

A Wheezing

0 = Absent
1 = Mild: expiratory wheezing on auscultation
2 = Moderate: dyspnea, inspiratory and expiratory wheezing
3 = Severe: dyspnea, use of accessory muscles, audible wheezing

---

IV **Gastrointestinal**

A Subjective complaints

0 = Absent
1 = Mild: itchy mouth, nausea, vomiting, abdominal pain, no change in activity
2 = Moderate: frequent complaints of nausea or pain, decreased activity
3 = Severe: child in bed; crying, notably distressed

B Objective complaints

0 = Absent
1 = Mild: 1 episode of emesis or diarrhoea
2 = Moderate: 2-3 episodes of emesis or diarrhoea, or 1 of each
3 = Severe: >3 episodes of emesis or diarrhoea or 2 of each

V **Cardiovascular**

A
0 = Absent: normal HR and/or BP for age or baseline
1 = Mild: colour change, subjective response (weak, dizzy), mental status change, tachycardia
2 = Moderate: drop in BP >20% from baseline
3 = Severe: CV collapse, signs of impaired circulation, unconsiousness, bradycardia

---

Child's Name:
Addressograph:

Date of Challenge: _____

Type of Challenge: _____

Please note that a challenge **should not be discontinued** until **2 objective symptoms** have been recorded.

Time	Dose	Symptom Score													
		% Rash	Pruritus	Urticaria	Rash	Sneezing	Nasal congestion	Runny nose	Larynx	Wheeze	GI subjective	GI Objective	CV	Total Score	Comments. Stop/Continue
Base	0														

Total Dose Ingested: _____ Time Stopped: _____ **Outcome:** Passed / Failed

Treatment given:

Ensure child remains on ward for 2 hours after the last symptoms have occurred. Children with a respiratory reaction should be discussed with a doctor or CNS and may need to stay for 4 hours.

Time:	Symptoms	Treatment	Comment

Discharge home. Time _____ Signature: Name: _____ Date: _____

Modified from Bock S A, Sampson H A, Atkins F M et al. *Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual.* J Allergy Clin Immunol 1988;82:986-97.

D Marriage, June 2016
Review date: June 2018

Appendix 7
Ethics, R&I and Study Sponsor Approval Letters

NHS
Health Research Authority
NRES Committee London - Camden & Islington
Room 002
Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne & Wear
NE32 3DT

Telephone: 0191 4283545

20 October 2014

Ms Deborah Marriage
Room 101, The Dug-Out, Lower Ground Floor
King David Building, Bristol Children's Hospital,
Upper Maudlin Street, Bristol
BS2 8BJ

Dear Ms Marriage

Study title:	**The NutCracker Study: A study of incidental sensitisation to peanut in egg allergic children, and the utility of component-resolved diagnostic testing to Ara h 2 in predicting clinical outcome. A Pilot Study.**
REC reference:	**14/LO/1894**
Protocol number:	**TBC**
IRAS project ID:	**133448**

Thank you for your e-mail correspondence of 20th October 2014. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 16 October 2014

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Participant consent form [Older children assent form]	1.1	16 October 2014
Participant information sheet (PIS) [Child information leaflet]	1.1	16 October 2014
Response to Request for Further Information		20 October 2014

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Covering letter on headed paper		15 July 2014
Evidence of Sponsor insurance or indemnity (non NHS Sponsors		09 July 2014

only)		
GP/consultant information sheets or letters	1.0	15 July 2014
Letter from sponsor		10 July 2014
Letters of invitation to participant	4.1	15 July 2014
Participant consent form [Consent form]	4.2	14 October 2014
Participant consent form [Older children assent form]	1.1	16 October 2014
Participant information sheet (PIS) [Parent information sheet (clean/tracked)]	4.2	14 October 2014
Participant information sheet (PIS) [Child information leaflet]	1.1	16 October 2014
REC Application Form [REC_Form_09102014]		09 October 2014
Research protocol or project proposal [October 2014]	4.2	
Response to Request for Further Information		20 October 2014
Response to Request for Further Information [e-mail correspondence - Deb Marriage]		14 October 2014
Summary CV for Chief Investigator (CI)		
Summary CV for supervisor (student research)		
Summary, synopsis or diagram (flowchart) of protocol in non technical language		

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

14/LO/1894 **Please quote this number on all correspondence**

Yours sincerely

H Hendn

Hayley Henderson
REC Manager

E-mail: nrescommittee.london-camdenandislington@nhs.net

Copy to: Professor Jane Millar, University of Bath

Karen Morgan, Research & Innovation Department

University Hospitals Bristol NHS
NHS Foundation Trust

Research and Innovation
University Hospitals Bristol NHS Foundation Trust
Education & Research Centre Level 3
Upper Maudlin Street
Bristol BS2 8AE

Tel 0117 342 0233
Fax 0117 342 0239

research@uhbristol.nhs.uk
http://www.uhbristol.nhs.uk/research-innovation

Ms Deb Marriage
Room 101, The DugOut
Lower Ground Floor, King David Building
Bristol Children's Hospital, Upper Maudlin St
BS2 8BJ

16/12/2014

NHS Permission for Research has been granted for the study detailed below at University Hospitals Bristol NHS Foundation Trust (UH Bristol). Permission is subject to any conditions and is effective from 16/12/2014 until 30/04/2016.

Dear Ms Deb Marriage

RE: The NutCracker Study: A study of incidental sensitisation to peanut in egg allergic children, and the utility of component-resolved diagnostic testing to Ara h 2 in predicting clinical outcome. A Pilot Study. R&D Number: CH/2014/4630

NHS permission for the above research has been granted on the basis of the application submitted and a favourable opinion from an authorised REC.

Permission is granted on the understanding that the study is conducted in accordance with the Research Governance Framework, Good Clinical Practice, and NHS Trust policies and procedures. As Principal Investigator it is your responsibility to ensure you and your team are familiar with relevant research related policies and procedures; these can be found at http://www.uhbristol.nhs.uk/media/2097744/research_policy_final_v0_7_21_02_14.pdf

It is also a condition of NHS Permission at this site that local recruitment data is uploaded to the EDGE system and the study record is kept up-to-date. Please contact the Research Management Office if you are unsure how to do this.

The following conditions must be met prior to recruitment commencing:

- A site file is set-up and delegation log established

UH Bristol is required to monitor research to ensure compliance with the Research Governance Framework and other legal and regulatory requirements. For further details about monitoring arrangements please contact the Research Management Office. The Research Management Office will monitor recruitment on an on-going basis and can provide support and advice if you are experiencing problems in meeting your targets within the agreed time frame.

The Research Management Office should be notified of any urgent safety measure taken in order to protect research participants against any immediate hazard to their health or safety. This should be within the same time frame as notification to the REC and any other regulatory bodies and should include the reasons why the measures were taken and any plan for further action.

NHS indemnity is provided for the period of permission given above. Requests for changes to the period of permission (e.g. an extension of the study) must be made to the Research Management Office before permission ceases with an explanation as to why the change is being sought.

All amendments (including changes to the local research team) need to be submitted in accordance with regulatory and national requirements which can be found on IRAS. Please note if we are sponsoring this study separate notification of an amendment already authorised by us as sponsor for submission to the regulatory bodies is not required, the sponsor authorisation will cover R&D acknowledgement of the amendment at this trust. The Research Management Office also needs to be notified if there are any changes to the study status.

We wish you every success with this study.

Yours sincerely,

Diana Benton
Head of Research and Innovation/Deputy Director of Research

Copy to:

Dr James Turner
Professor Jane Millar

Professor Jane Millar OBE
Pro-Vice-Chancellor Research

Vice-Chancellor's Office
Bath BA2 7AY
Tel: 01225 386141
Email: Pro-vc-research@bath.ac.uk

Deborah Marriage
Department for Health

9 July 2014

Dear Deborah,

The NutCracker Study: A study of incidental sensitisation to peanut in egg allergic children, and the utility of component-resolved diagnostic testing to Ara h 2 in predicting clinical outcome.

I am pleased to confirm that the University is prepared to act as sponsor under the Department of Health's Research Governance for Health and Social Care (2005) subject to the following:

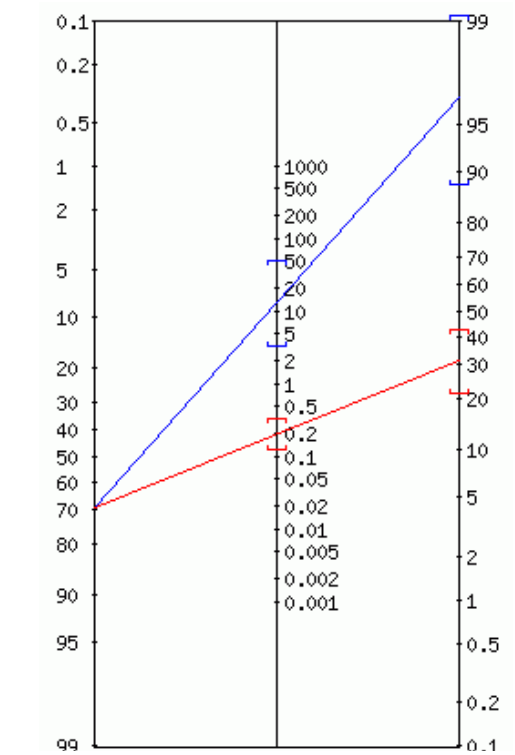
1. The University requires you, as Chief Investigator, to conduct the study in compliance with the requirements of the Framework so it is able to meet its obligations as sponsor.
2. University professional indemnity and insurance will apply to the study as appropriate, within the UK.
3. As the Chief Investigator for the study, the University requires you to comply with the University policy on research data and all systems of good practice.
4. Substantial amendments and reports should be submitted to the undersigned.

Yours sincerely

Professor Jane Millar
Pro-Vice-Chancellor

Appendix 8

Fagan's Nomogram for post-test probability of having peanut allergy for egg-allergic children with a positive Ara h 2-specific IgE concentration attending the tertiary paediatric allergy clinic



Pre-test probability Likelihood ratio Post-test probability

\+ Test Post-test probability

\- Test Post-test probability